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1. SHAPIRO, S., WEINER, M., ET AL.: AM. HEART J. 40:766 (NOV.) 1950.

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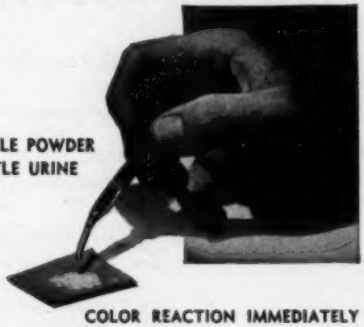

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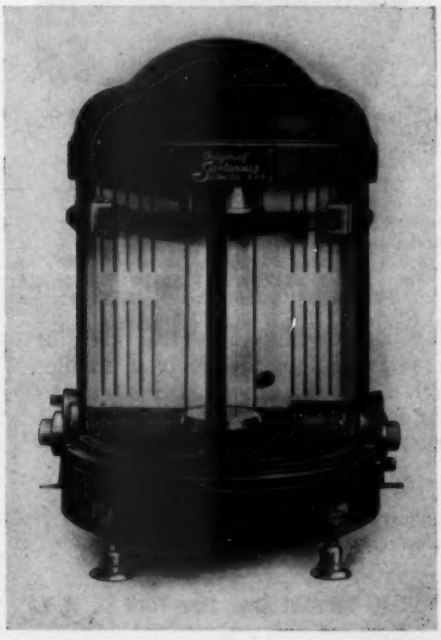

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EFFECT OF HYPERVITAMINOSIS A AND HYPOVITAMINOSIS A ON THE SKELETON OF A DUCK

R. H. RIGDON, M.D.
J. C. RUDE
AND
J. G. BIERI
GALVESTON, TEXAS

THE MECHANISM of the neurologic manifestations in vitamin-A-deficient animals has been frequently discussed [1] without any one presenting an opinion acceptable to all others, as indicated by a recent review.[2] Wolbach and Bessey [1c] expressed the opinion that a retardation of skeletal growth occurs in vitamin-A-deficient animals, whereas the nervous system continues to grow at its normal rate. This results in a distortion of the nervous tissue in the vertebral canal and in the cranial cavity, producing the neurologic manifestations. Adamstone [3] observed that the brains of chicks fed a vitamin-A-deficient ration were smaller than those of normal birds of the same age but attributed this variation to the slower growth of the former. Recently Fletcher and Rigdon [4] reported their observations on the neurologic lesions in ducks fed a vitamin-A-deficient ration. The latter investigators failed to observe a disproportion in the rates of growth of the nervous and osseous systems in the duck but described foci of degeneration and bony metaplasia

From the Laboratory of Experimental Pathology and the Departments of Radiology and Biochemistry, University of Texas Medical Branch.

This investigation was supported by a research grant from the National Institutes of Health, Public Health Service. We wish to acknowledge the following: The 50 unit capsules and a portion of the 10,000 unit capsules of vitamin A were supplied by Eli Lilly & Company; the soybean oil was contributed through the courtesy of Mr. L. V. Curtin, of the Buckeye Cotton Oil Company; the "Delsterol" was supplied by Mr. George H. Man of E. I. Dupont de Nemours & Company; Dr. H. C. Schaefer, of the Ralston Purina Company, kindly prepared the vitamin A-deficient ration No. 2; Dr. J. R. Couch, of Texas A. and M. College, assisted in the preparation of the deficient ration No. 1; Drs. Charles E. Dunlap and William H. Sternberg, of the department of Pathology of Tulane University, reviewed the histologic sections of bones.

1. (*a*) Mellanby, E.: The Experimental Production and Prevention of Degeneration in the Spinal Cord, Brain **54**:247, 1931. (*b*) Zimmerman, H. M.: Lesions of the Nervous System in Vitamin Deficiency: Rats on a Diet Low in Vitamin A, J. Exper. Med. **57**:215, 1933. (*c*) Wolbach, S. B., and Bessey, O. A.: Tissue Changes in Vitamin A Deficiency, Physiol. Rev. **22**:233, 1942; (*d*) Vitamin A Deficiency and the Nervous System, Arch. Path. **32**:689 (Nov.) 1941.

2. Neurologic Manifestations Associated with Vitamin A Deficiency in Young Ducks, editorial, Nutrition Rev. **7**:188, 1949.

3. Adamstone, F. B.: Histologic Comparison of the Brains of Vitamin A-Deficient and Vitamin E-Deficient Chicks, Arch. Path. **43**:301 (March) 1947.

4. Fletcher, D. E., and Rigdon, R. H.: Neurologic Manifestations Associated with Vitamin A Deficiency in Young Ducks, Arch. Neurol. & Psychiat. **61**:199 (Feb.) 1949.

within the nervous system. Wolbach [5] more recently expressed the opinion that the neurologic manifestations in vitamin-A-deficient animals are the result of the effect of the lack of this vitamin on skeletal growth. He stated that "in the chick,[6] and therefore presumably in the duck, the effects of vitamin deficiency on skeletal growth are similar to those occurring in rats, guinea pigs and dogs."

The present investigation was instituted to study the effects of hypervitaminosis and hypovitaminosis A on the skeleton of the duck.

EXPERIMENTAL PROCEDURE

Three hundred white Pekin ducks from 0 days to 12 weeks of age were used. They were obtained from one commercial hatchery and were approximately 48 hours of age when received. The birds were kept in standard batteries, with food and water available at all times. These experiments extended over approximately two years.

Three rations were used. The standard ration consisted of commercial preparations, the specific composition of which is unknown.[7] Vitamin-A-deficient ration No. 1 was a synthetic ration which contained the following:

Casein (crude)	18%
Gelatin	10%
Soybean oil	10%
Methionine	0.3%
Dextrose	57%
Calcium carbonate	12.0 gm./kg.
Potassium phosphate, dibasic	13.0 gm./kg.
Calcium phosphate, dibasic U. S. P.	10.0 gm./kg.
Magnesium sulfate	4.0 gm./kg.
Sodium chloride	6.7 gm./kg.
Ferric citrate U. S. P.	1.1 gm./kg.
Potassium iodide U. S. P.	0.03 gm./kg.
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Choline chloride	2,000.0 mg./kg.
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Menadione U. S. P.	0.5 mg./kg.
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* Association of Official Agricultural Chemists.
† This is a DuPont product.

Vitamin-A-deficient ration No. 2 was prepared by the Ralston Purina Company and contained the following:

Mineralized salt	0.25%
Calcium carbonate	2.00%
Wheat germ	5.00%
Soybean-oil meal	18.00%
Fish meal (60%)	6.00%
Meat scraps	4.00%
Brewers' yeast	5.00%
Ground wheat	19.65%
White corn	29.00%
Ground oats	5.00%
Molasses	5.00%
Dry vitamin D (2,000 U. S. P. units vitamin D)	0.10%
Purifiavin (250)® *	1.00%
Thiamine hydrochloride U. S. P.	0.5
Riboflavin U. S. P.	0.5
Choline chloride	200.0
Folic acid	0.5
Nicotinic acid U. S. P.	5.0

* Purifiavin® is a mixture of B vitamins as follows: mg. per 100 Gm. of ration

5. Wolbach, S. B.: Letter to the Editor, Nutrition Rev. **7**:319, 1949.

6. Wolbach, S. B.: Vitamin-A Deficiency and Excess in Relation to Skeletal Growth, J. Bone & Joint Surg. **29**:171, 1947.

7. The preparations used were those made and marketed by the Ralston Purina Company, St. Louis, under the names duck startena® and growena.®

The vitamin-A preparations used to supplement the rations were commercial ones[8] and were given by mouth either as a single dose or as multiple doses, depending on the total amount to be given during a specific interval. The total amount of vitamin A given per day varied as shown in the different experiments. Ducks given 100,000 and 200,000 units had usually been given smaller amounts at first and the dose was rapidly increased to reach this large daily dose.

Two hundred sixty roentgenograms were made. One hundred and ten of these were of one extremity only. The ducks were killed at ages varying from 1 day to 7 weeks. A femur with tibia and fibula attached was disarticulated, and the skin was removed. The bones with their attached muscles were fixed in dilute formaldehyde solution U. S. P. (1:4) for 24 hours to 20 days preceding the time the roentgenograms were made. One hundred and fifty roentgenograms were made of the entire bodies of 24 ducks. These were made at five- to seven-day intervals over a period of six weeks. The radiologist made interpretations of all the roentgenograms without having any information as to the specific rations the birds had received.

Pathologic studies were made of one femur, including both ends, from each of 124 ducks. In a few instances cross sections of the femur were taken for histologic study. The femur was removed immediately after the bird was killed. The muscles were dissected, and the bone was divided longitudinally and fixed either in Bouin's solution or formaldehyde solution U. S. P. diluted 1:4. Paraffin sections were prepared and stained with hematoxylin and phloxine. In

TABLE 1.—*Vitamin-A and Carotene Contents of Serums and Livers of Ducks on Standard Ration and Standard Ration Supplemented with Vitamin A*

Ducks	Age, Days	Daily Supplement, U. S. P. (I. U.) Units	Serum		Liver	
			Vitamin A	Carotene	Vitamin A	Carotene
11	34-72	None	59.1 (38.4-83.3)*	286 (153-420)	7.3 (3.4-16.6)	4.2 (0.8-7.5)
8	27-50	25,000	141 (93-177)	227 (136-312)	790 (445-1,129)	4.3 (2.1-6.2)
6	29†	100,000	108 (57.7-179)	42 (24.6-54)	1,133 (888-1,444)	5.2 (3.6-8.3)

* Variations observed within the group are given in parentheses.
† These ducks were given 25,000 units of vitamin A from the 4th to the 9th experimental day. The dose was increased to 30,000 units on the 10th day, to 55,000 units on the 16th day, to 75,000 units on the 26th day, and to 100,000 units on the 33d day. This level was continued for the remainder of the experiment.

many instances one femur was used for the histologic preparations, while the other was used for the roentgenogram.

Carotene and vitamin-A assays were made on the serums and the livers of 25 ducks. The ration fed and the age when these birds were killed are shown in Table 1. Food and water were withdrawn 24 hours before the ducks were killed. Blood was obtained from the heart. After it had clotted it was centrifuged, and the serum was removed and stored at 5 C. for 24 to 72 hours. The liver was removed, and the blood on the external surface was absorbed on filter paper. The liver was then stored at —15 C. Vitamin A was determined by the Carr-Price color reaction,[9] and the carotene was determined by its absorption at 450 millimicrons. Either 5 to 10 ml. of serum was saponifed with an equal volume of normal alcoholic potassium hydroxide and extracted three times with hexane. Aliquots of the hexane were taken for the carotene estimation, then evaporated to dryness *in vacuo*, and the residue was dissolved in chloroform for the vitamin-A determination. The liver was chopped while frozen and digested

8. The preparations were alphalin,® in capsules containing 10,000 U. S. P. units, made by Eli Lilly & Company, and super A® concentrate, in capsules containing 25,000 units, made by The Upjohn Company.

Special gelatin capsules containing 50 units of vitamin A were supplied by Eli Lilly & Company.

9. Carr, F. H., and Price, E. A.: Colour Reactions Attributed to Vitamin A, Biochem. J. **20**:497, 1926.

with 4N alcohol potassium hydroxide. Aliquots were extracted and the determinations carried out as with the serum.

The birds were carefully observed during the time of this experiment for the occurrence of abnormal clinical symptoms. Some ducks were weighed at daily intervals.

EFFECTS OF HYPOVITAMINOSIS A AND HYPERVITAMINOSIS A ON THE WEIGHT AND THE COLOR OF YOUNG DUCKS

The weights of ducks fed the standard ration supplemented with vitamin A and the two vitamin-A-deficient rations, Nos. 1 and 2, supplemented with vitamin A are shown as recorded during the first 21 days of life in Figure 1. Ducks fed the standard ration supplemented daily with 10,000 units of vitamin A weighed slightly less than the controls. Those given 100,000 units of vitamin A a day, increased to 200,000 units by the fifth day of the experiment, gained at a much slower rate than the birds on the standard ration. Birds fed vitamin-A-deficient ration No. 2 and those fed this ration supplemented daily with 50 and 10,000 units of vitamin A gained at approximately the same rate during the first 12 days. However, after this time those receiving the vitamin-A supplement gained at a much faster rate than those not given vitamin A. Each of 10 ducks fed this vitamin-A-deficient ration (No. 2) was dead by the 21st experimental day. It is of interest to observe that the ducks fed this vitamin-A-deficient ration supplemented with vitamin A gained at approximately the same rate as the birds fed the standard ration.

Ten ducks fed vitamin-A-deficient ration No. 1 gained very little during the first 16 days of the experiment, at which time all were dead. The birds given this A-deficient ration (No. 1) and 75,000 units of vitamin A per day failed to gain at a rate equal to those given the same A-deficient ration and 10,000 and 25,000 units of vitamin A per day (Fig. 1). Before death, the ducks fed vitamin-A-deficient ration No. 1 became progressively weaker and showed some of the neurologic symptoms previously described in vitamin-A-deficient ducks.[10]

It is obvious from these experiments that large amounts of vitamin A decrease the rate of gain in weight. Fifty units of vitamin A given daily to ducks on vitamin-A-deficient ration No. 2 apparently is adequate for normal growth during the first three weeks of life. In subsequent experiments it was observed that ducks fed this vitamin-A-deficient ration and given 50 units of vitamin A daily failed to gain at a normal rate after approximately the 30th day of life.

To observe the effect of a large quantity of vitamin A on ducks fed the standard ration, 10 birds, 2 days of age, averaging 63.2 gm. in weight, were given 100,000 units of vitamin A by mouth per day for three days, at which time they averaged 88.4 gm. The daily dose of vitamin A then was increased to 150,000 units each day for two days, at which time the average weight was 111.8 gm. At this time the amount of vitamin A was increased to 200,000 units per day. On the 13th day of the experiment the ducks averaged 192.2 gm. and were receiving 200,000 units of vitamin A. No abnormal clinical manifestations were observed during this period when they were receiving from 1,000 to 1,800 units of vitamin A per gram of body weight. Some of these ducks continued to receive 200,000 units of vitamin A for

10. Ridgon, R. H.: Clinical Observations on Vitamin A Deficiency in Young Ducks, Texas Rep. Biol. & Med. 5:438, 1947.

three weeks without showing any abnormal symptoms except for the color of their skin. When 1 month of age the average weight of these birds was 934 gm.

During the time of this experiment in which large amounts of vitamin A were given to ducks fed a standard ration, it was noted that the skin of the feet and legs and of the bills was much lighter in color than that of the controls fed the standard ration for a similar period. To observe this change further, 10 ducks were given the standard ration and 100,000 units of vitamin A per day. After 30 days the color of their feet, legs and bills was much lighter than that of the controls fed the standard ration.

To determine whether this decrease in the orange color of the extremities and the bills of ducks given large amounts of vitamin A was reversible, five ducks were

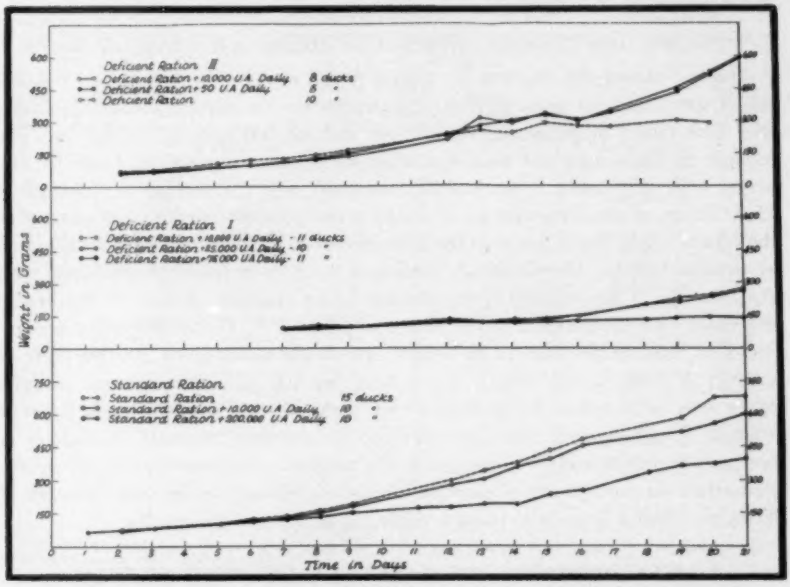
Deficient Ration II
Deficient Ration + 10,000 U.A. Daily 8 ducks
Deficient Ration + 50 U.A. Daily 5 "
Deficient Ration 10 "
Deficient Ration I
Deficient Ration + 10,000 U.A. Daily - 11 ducks
Deficient Ration + 25,000 U.A. Daily - 10 "
Deficient Ration + 75,000 U.A. Daily - 11 "
Standard Ration
Standard Ration 15 ducks
Standard Ration + 10,000 U.A. Daily 10 "
Standard Ration + 300,000 U.A. Daily 10 "
Weight in Grams
Time in Days

Fig. 1.—The average weights of groups of ducks fed the standard ration and the two vitamin-A-deficient rations are shown in this graph. The weights of groups fed these rations supplemented with vitamin A are also included. Ducks given either a hypovitaminosis-A or a hypervitaminosis-A ration usually gain at a slower rate than birds on the standard ration. The effect of hypervitaminosis A on the weight curve of ducks fed a standard ration is shown nicely in this graph. Ducks failed to gain when fed vitamin-A-deficient ration No. 1, similar to the birds fed vitamin-A-deficient ration No. 2. The rate at which the vitamin-A supplement was increased in the experiment is given.

placed on the standard ration supplemented with 10,000 units of vitamin A for three days. At this time the amount of vitamin A was increased to 20,000 units per day. After seven days the amount of vitamin A was increased to 40,000 units per day, and the following day it was increased to 50,000 units per day. Eight days later the dose was increased to 75,000 units, seven days later to 100,000 units, and four days later to 125,000 units. The latter amount was given for 10 days more, at which time the birds were 38 days of age. The coloring in the extremities and

bills was definitely less than that of the controls when observed on the 24th day of this experiment. This diminution of color persisted until the 38th day, at which time the vitamin-A supplementation was discontinued. The birds were fed the standard ration for two more months, at which time the coloring in the bill was still less than that of the controls; however, there was little variation in the color of the extremities in the two groups. These ducks, like others given large amounts of vitamin A, did not gain as rapidly as the controls. However, when the vitamin A was withdrawn, they gained at a more rapid rate and appeared perfectly normal. The extremities and the bills of all ducks fed the vitamin-A-deficient rations, Nos. 1 and 2, were pale in color. The coloring in these vitamin-A-deficient birds was similar to that in the ducks given the standard ration supplemented with 100,000 to 200,000 units of vitamin A.

VITAMIN-A AND CAROTENE CONTENTS OF SERUMS AND LIVERS OF DUCKS

Table 1 shows the amounts of vitamin A and carotene in micrograms per 100 ml. of serum and per gram of liver in ducks fed the standard ration and birds fed this same ration supplemented with 25,000 and 100,000 units of vitamin A. The number of ducks used and their age when killed are also given in Table 1. The serum level of vitamin A is markedly increased over the normal in ducks given 25,000 units of vitamin, whereas in ducks given 100,000 units it is elevated over the control levels, but is less than the levels observed in ducks receiving 25,000 units of vitamin A daily. The vitamin-A content of the liver increases progressively with the increase of the vitamin-A supplement. The carotene content of the serum decreases with the increase in the intake of vitamin A. In the controls there are 286 γ of carotene per 100 ml. of serum, and in the ducks given 100,000 units of vitamin A there is only 42.0 γ of carotene per 100 ml. The carotene contents of the livers of the same ducks show a very slight increase with the larger doses of vitamin A as compared with the controls. The increase in intake of vitamin A produces an increase in liver storage of this vitamin but apparently has no significant effect on the amount of carotene deposited. Owing to the small number of birds, no attempt is made to make a statistical analysis of the results.

ROENTGENOGRAPHIC OBSERVATIONS ON THE BONES IN THE EXTREMITIES OF DUCKS

Roentgenograms were made of the femurs, the tibias and the fibulas of 110 ducks (Figures 2, 3, and 4). The ration and the vitamin-A supplement that each bird received and the age when killed are shown in Table 2. No variations were observed in the ducks fed the standard ration when these ducks were compared with those fed the same ration supplemented with either 10,000 or 100,000 units of vitamin A for 35 and 49 days. Furthermore, no differences were noted in the ducks fed the standard ration and the same ration supplemented with 200,000 units of vitamin A for 30 days. Two birds fed the standard ration for 30 days showed what was interpreted as transverse lines of arrested growth. No significant variations were observed in the roentgenograms of any ducks younger than the above fed either the standard ration or the standard ration supplemented with varying amounts of vitamin A.

The bones usually were smaller in the older birds fed the vitamin-A-deficient rations when compared with those from birds fed either the standard ration or the deficient rations supplemented with vitamin A. Some of the ducks fed vitamin-A-deficient ration No. 2 for 14 days showed a little fuzziness at the proximal end of the tibia. However, similar changes were present in birds of equal age fed the same deficient ration supplemented with 10,000 units of vitamin A. Some of the ducks fed vitamin-A-deficient ration No. 2 supplemented with 50 units of vitamin A for 29 and 38 days showed some demineralization of the upper end of the tibia. Other birds fed the same ration supplemented with 10,000 units of vitamin A showed no changes. Therefore, it seems to us that the changes observed

TABLE 2.—*The Number and the Age of Ducks on Which Histologic and Roentgenologic Studies Were Made, and the Rations and Vitamin-A Supplements That Were Given*

Ducks					Ducks				
Studied Roentgenologically	Studied Histologically	Age, Days	Ration	Vitamin-A Supplement, Units per Day	Studied Roentgenologically	Studied Histologically	Age, Days	Ration	Vitamin-A Supplement, Units per Day
5	5	0-2	Standard	0	2	2	7	A-deficient #1	0
4	4	7-10	Standard	0	0	5	15-19	A-deficient #1	0
2	5	13-15	Standard	0	2	3	21	A-deficient #1	0
2	4	21	Standard	0	2	2	7-8	A-deficient #1	50
0	8	25-28	Standard	0	4	4	14-19	A-deficient #1	50
4	2	29-30	Standard	0	2	2	7-8	A-deficient #1	10,000
5	0	35-38	Standard	0	2	5	14-16	A-deficient #1	10,000
1	9	45-49	Standard	0	4	4	19-21	A-deficient #1	10,000
3	3	21	Standard	10,000	3	5	28-30	A-deficient #1	10,000
3	3	28	Standard	10,000	2	2	7-8	A-deficient #1	25,000
4	0	35-38	Standard	10,000	4	4	14-19	A-deficient #1	25,000
0	3	49	Standard	25,000	3	3	28	A-deficient #1	25,000
2	2	7	Standard	200,000*	2	2	7-8	A-deficient #1	50,000
2	2	14	Standard	200,000*	4	4	14-19	A-deficient #1	75,000†
2	2	21	Standard	200,000*	3	3	28	A-deficient #1	75,000†
4	2	29-30	Standard	200,000*	7	5	14-20	A-deficient #2	0
					2	0	23	A-deficient #2	0
					2	2	29	A-deficient #2	50
					2	0	38	A-deficient #2	50
					5	5	14-21	A-deficient #2	10,000
					7	2	28-30	A-deficient #2	10,000
					2	0	38	A-deficient #2	10,000

* 100,000 units of vitamin A was given from two to five days, 150,000 units from six to seven days, and 200,000 units during the remaining time.
† 50,000 units of vitamin A was given from one to eight days and 75,000 units during the remaining time.

in some of the ducks fed the vitamin-A-deficient ration with or without a supplement of vitamin A were not related specifically to vitamin A.

Since only one roentgenogram was made, at the time of death, in the above experiments, a series of roentgenograms were made at 3- to 11-day intervals over a period of 46 days on a group of 12 ducks fed the standard ration. A total of 72 roentgenograms was made on this group of birds between the ages of 2 and 46 days. A similar procedure was carried out on six ducks fed the standard ration supplemented with 25,000 units of vitamin A per day and on six ducks fed the same ration supplemented with 25,000 units beginning on the fourth day of the experiment. This dosage was increased to 30,000 units on the 10th day, to 55,000 units on the 16th day, to 75,000 units on the 26th day, and to 100,000 units for the remaining time of this experiment. A total of 78 roentgenograms were made on these two hypervitaminosis-A groups of ducks between the ages of 7 and 45 days.

Few variations were observed in the roentgenograms of the ducks fed the standard ration. Sometimes the proximal end of one tibia showed a greater density than the corresponding area of the opposite tibia. Sometimes there appeared to be some demineralization of the head of the tibia. However, the roentgenogram a week later failed to show the change. The roentgenogram on one duck 45 days of age fed the standard ration showed a marked variation in the density of the bone at the proximal end of each tibia. This, of course, suggested to us either an irregular

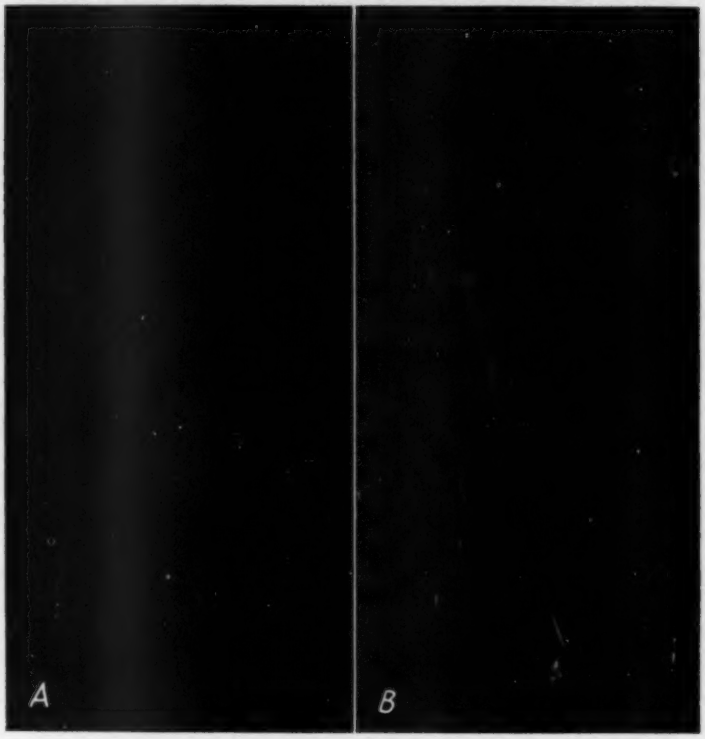

Fig. 2.—*A*, femur and tibia of Duck 63, which was fed the standard ration for 49 days. *B*, femur and tibia of Duck 102, which was fed the standard ration supplemented daily with vitamin A as follows: 100,000 units the first three days; 150,000 units on the fourth and fifth day; 200,000 units on the sixth day and throughout the remaining time of the experiment. No significant variations are observed in the bones of these two ducks except that those of Duck 102 are slightly smaller than those of Duck 63.

laying-down of calcium or some sclerosis within the marrow cavity. However, since this lesion occurred in a duck fed the standard ration, we do not consider it related to either hypervitaminosis or hypovitaminosis A.

Roentgenograms of the ducks fed the standard ration supplemented with 25,000 units of vitamin A showed few variations within the group and no significant

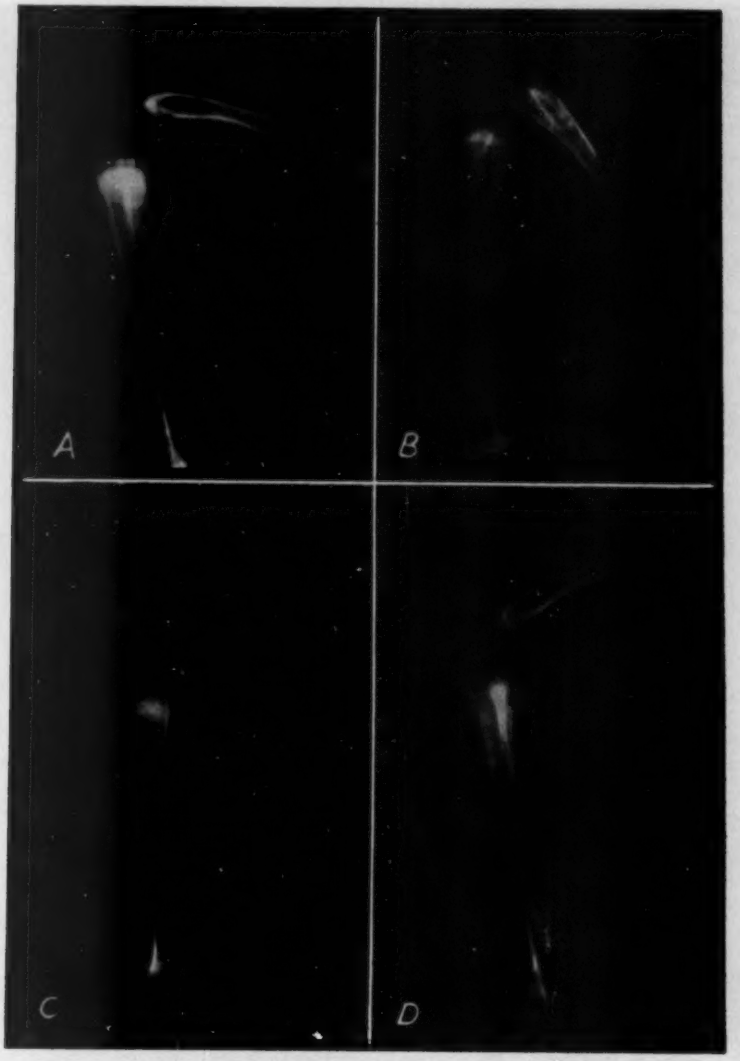

Fig. 3.—*A*, femur and tibia of Duck 34, which was fed a vitamin-A-deficient ration, No. 1, for 21 days. *B*, femur and tibia of Duck 38, which was fed the same vitamin-A-deficient ration supplemented with 10,000 units of vitamin A daily for 21 days.

C, femur and tibia of Duck 73, which was fed a vitamin-A-deficient ration, No. 2, for 20 days. *D*, femur and tibia of Duck 108, which was fed the same vitamin-A-deficient ration supplemented with 10,000 units of vitamin A daily for 21 days.

No significant variation is present in *A*, *B*, and *D*. The bones in *C* are smaller than those in *D*. The fuzziness at the end of the tibia in *C* is typical of that normally found in younger ducks fed a standard ration.

differences from ducks of corresponding ages fed only the standard ration. The roentgenograms of the birds fed the standard ration supplemented with 25,000 units increased to 100,000 units of vitamin A likewise did not show any variations as compared with the controls. The same demineralization of the end of the tibia was present in some of the ducks in the latter, hypervitaminosis-A group as observed in those fed the standard ration. When the roentgenograms of the 12 ducks fed the standard ration were compared with the roentgenograms of the 12 ducks fed the same ration supplemented with 25,000 and 100,000 units of vitamin A, no variations were observed that in our opinion could be attributed to the effect of hypervitaminosis on the bones in the extremities of the duck.

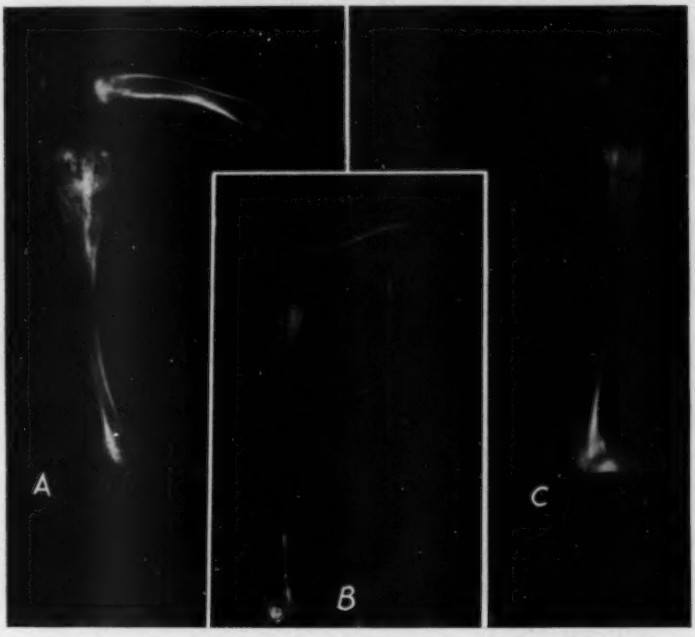

Fig. 4.—*A*, femur and tibia of Duck 33, which was fed a vitamin-A-deficient ration, No. 1, for 21 days. *B*, femur and tibia of Duck 61, which was fed the standard ration for 20 days. *C*, femur and tibia of Duck 87, which was fed a vitamin-A-deficient ration, No. 2, for 20 days. *B* and *C* are similar. *A* differs from these in that the proximal end of the tibia shows an uneven density of calcification.

PATHOLOGIC STUDY

As shown in Table 2, the femurs of 37 ducks ranging in age from 0 to 49 days of age and fed the standard ration were studied to establish the normal (Figs. 5 and 6). Sections from the femurs of 17 ducks fed the same ration supplemented with vitamin A and killed at corresponding intervals were compared with the normal. No significant differences were observed (Fig. 7). In a similar manner, femurs from ducks fed the two vitamin-A-deficient rations and these same rations supplemented with vitamin A were compared (Fig. 8). No significant variations

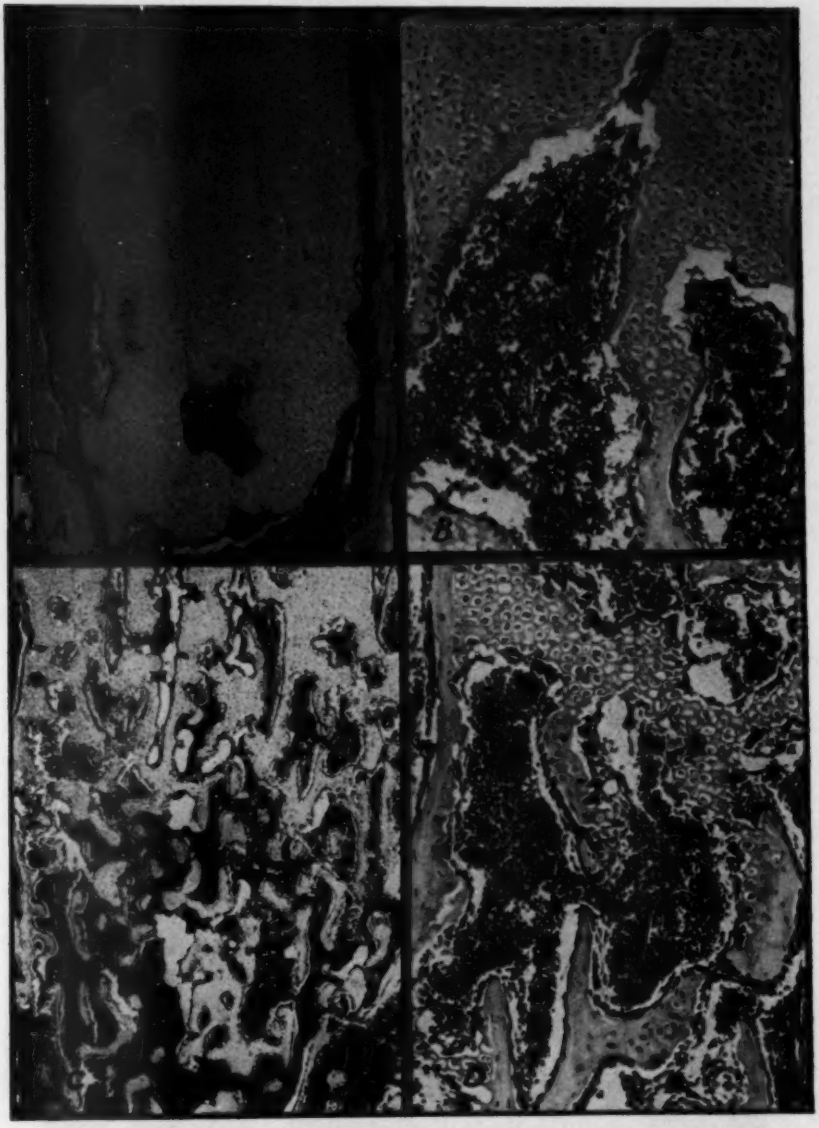

Fig. 5.—*A*, section of femur of Duck 5, aged 3 days, fed standard ration; hematoxylin and phloxine stain; × 31. *B*, same as *A;* hematoxylin and phloxine stain; × 110. *C*, section of femur of Duck 8, aged 10 days, fed standard ration; hematoxylin and phloxine stain; × 31. *D*, same as *C;* hematoxylin and phloxine stain; ×110. *A*, *B*, *C*, and *D* show the histologic characteristics of the femurs of normal ducks at 3 and 8 days of age. Figure 6 shows the histologic characteristics of the femurs of normal ducks at 15 and 27 days of age.

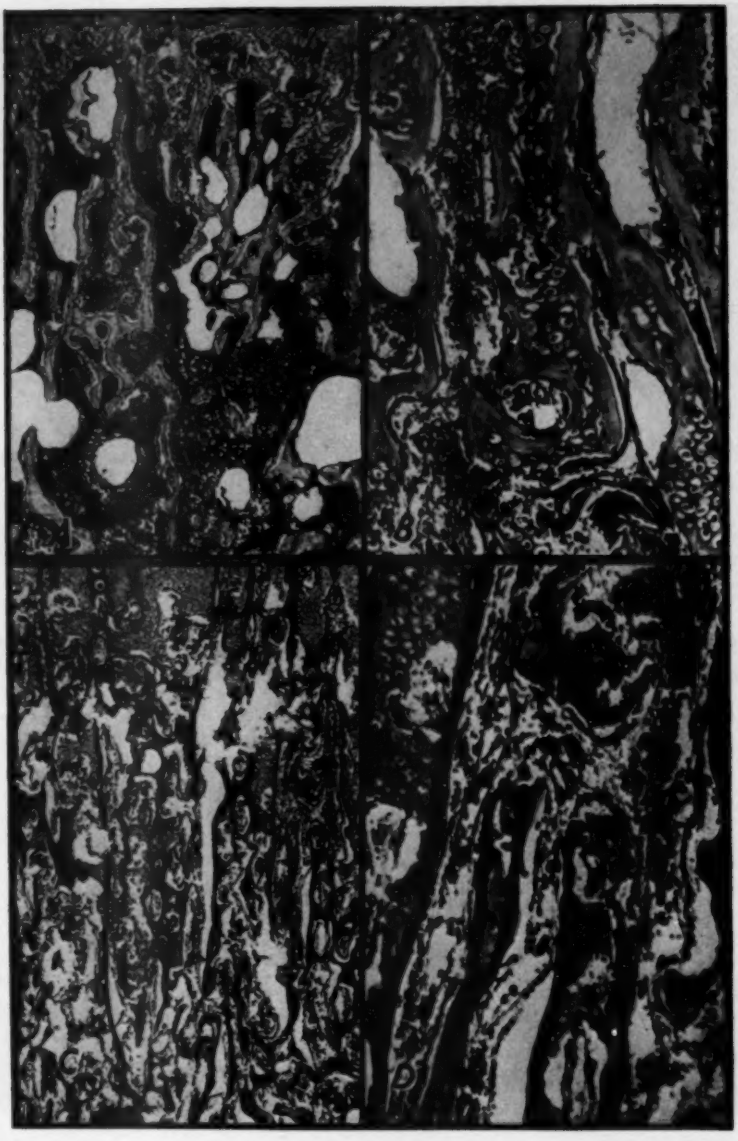

Fig. 6.—*A*, section of femur of Duck 168, aged 15 days, fed standard ration; hematoxylin and phloxine stain; × 31. *B*, same as *A;* hematoxylin and phloxine stain; × 110. *C*, section of femur of Duck 149, aged 27 days, fed standard ration; hematoxylin and phloxine stain; × 31. *D*, same as *C;* hematoxylin and phloxine stain; ×110. *A*, *B*, *C*, and *D* show the histologic characteristics of the femurs of normal ducks at 15 and 27 days of age. Figure 5 shows the femurs of normal ducks at younger ages.

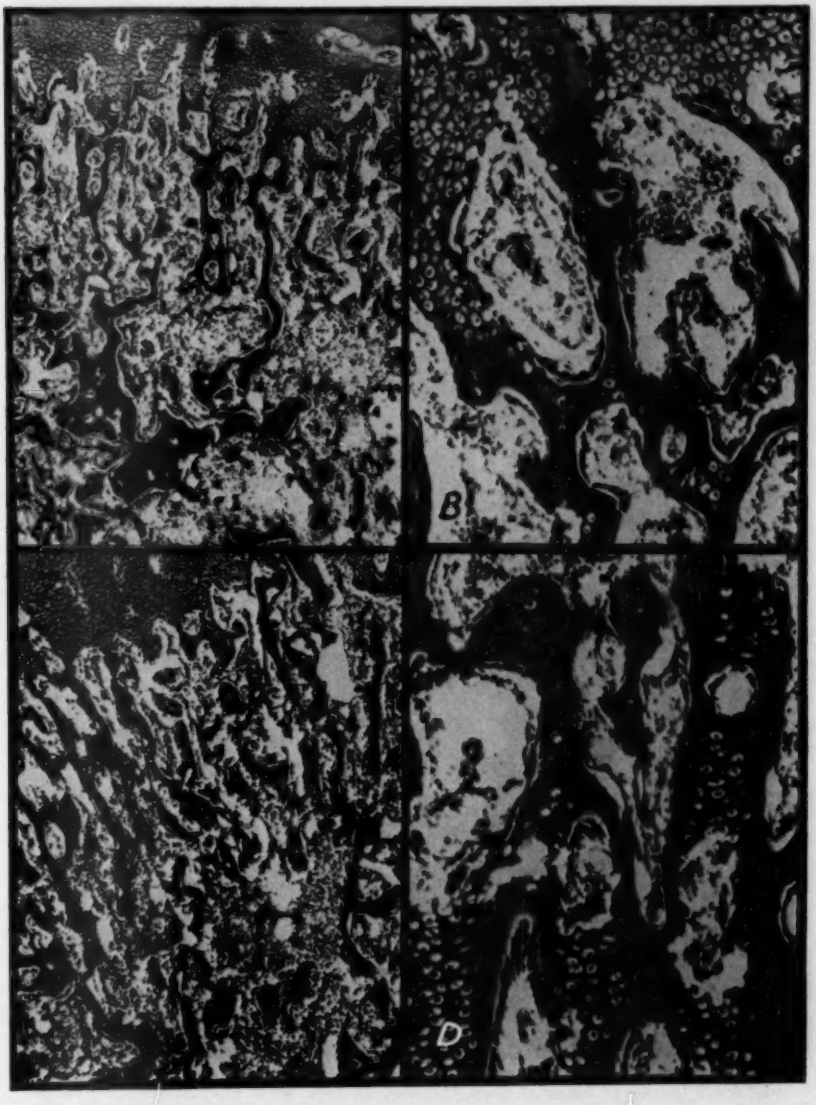

Fig. 7.—*A*, section of femur of Duck 85, aged 45 days, fed standard ration; hematoxylin and phloxine stain; × 31. *B*, same as *A*; hematoxylin and phloxine stain; × 110. *C*, section of femur of Duck 92, aged 41 days, fed standard ration supplemented with vitamin A; hematoxylin and phloxine stain; × 31. The supplement was 25,000 units from the 4th to the 9th experimental day; it was increased to 30,000 units on the 10th day, to 55,000 units on the 16th day, to 75,000 units on the 26th day and to 100,000 units on the 33d day. This level was continued for the remainder of the experiment. *D*, same as *C*; hematoxylin and phloxine stain; × 110. There is no histologic difference in the bones of Duck 92, given large amounts of vitamin A, and Duck 85, fed only the standard ration.

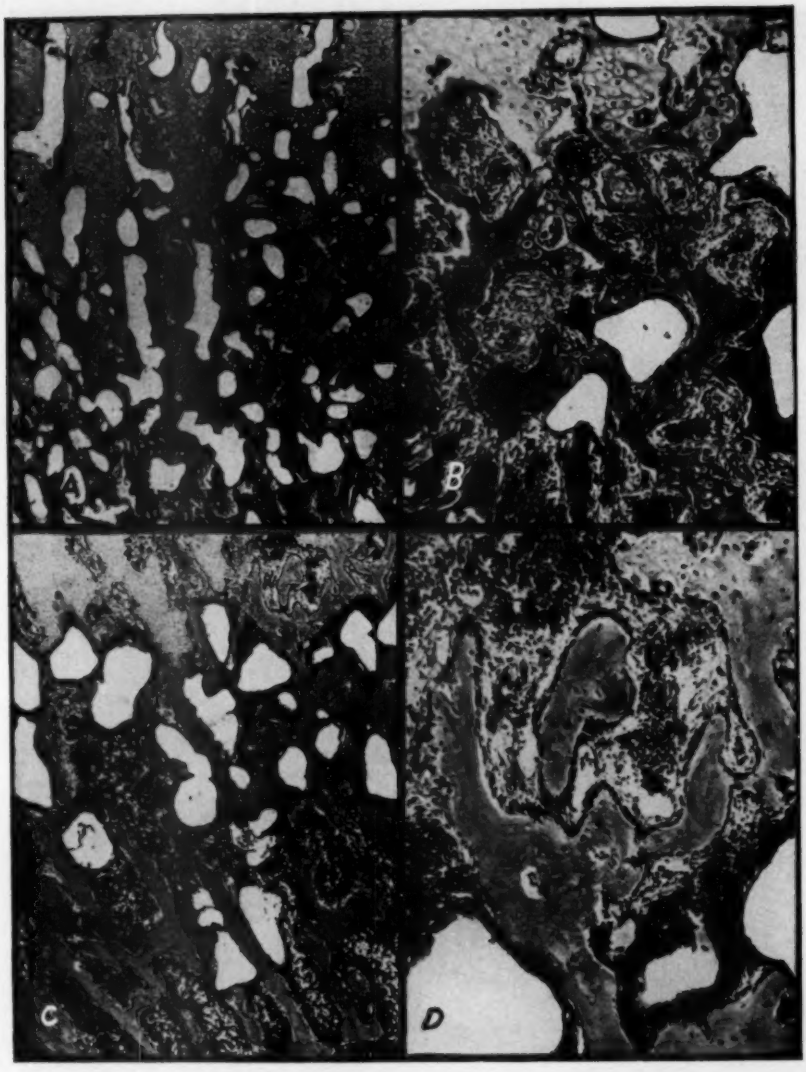

Fig. 8.—*A*, section of femur of Duck 152, aged 19 days, fed a vitamin-A-deficient ration, No. 1; hematoxylin and phloxine stain; × 31. *B*, same as *A*; hematoxylin and phloxine stain; × 110. *C*, section of femur of Duck OF-4, aged 19 days, fed the same ration, No.1, supplemented with 50 units of vitamin A; hematoxylin and phloxine stain; × 31. *D*, same as *C*; hematoxylin and phloxine stain; × 110. There is no significant histologic variation between *A* and *B* and *C* and *D*. This amount of vitamin A is adequate for normal growth at this age. There appears to be more of osteoid tissue and osteoblasts in these ducks than is present in ducks 15 days of age that were fed the standard ration, as shown in Figure 5*A* and *B*. This variation, however, is not a typical feature of all birds fed this deficient ration.

were observed except for the differences in the size of the bones. The bones of those ducks which were fed the vitamin-A-deficient rations frequently were smaller when compared with those from either the ducks fed the same vitamin-A-deficient ration supplemented with vitamin A or those fed the standard ration. As shown earlier in this study, the ducks receiving the vitamin-A-deficient rations weighed less than birds of corresponding age fed the standard ration.

The cartilage that constitutes the major portion of the femur in the newly hatched duck was rapidly replaced by trabeculae of osteoid tissue (Figs. 5 and 6). There did occur some variation in the number of osteoblasts and osteoclasts in different birds. There was also a marked histologic variation in different areas of the end of the same femur. However, at no time could this be correlated with specific rations. Some of the ducks given vitamin-A-deficient ration No. 1 showed a larger number of fibroblasts between the trabeculae than others. However, this was not a constant histologic finding. It is suggested that the difference in the microscopic picture of some of the ducks fed deficient ration No. 1 is within the range of normal or is the result of a factor other than a deficiency of vitamin A, since some ducks fed this same deficient ration and given 50 units of vitamin A showed similar histologic features (Fig. 8).

Cross sections of femurs from five ducks fed the standard ration supplemented with 200,000 units of vitamin A were studied. The femoral structure of these hypervitaminotic birds was similar to that of a duck fed the standard ration with no supplement and also that of a duck fed vitamin-A-deficient ration No. 1.

COMMENT

The duck, like other experimental animals, fails to gain in weight when placed on a ration with either a deficiency or an excess of vitamin A. The clinical manifestations of hypervitaminosis A in the duck are essentially nil as compared with those in the rat as reported by Moore and Wang.[11] Furthermore, no fractures were observed in any of our birds given as much as 200,000 units of vitamin A. Wolbach and Bessey [1d] reported the occurrence of fractures "in rats given excessive amounts of vitamin A." Furthermore, Wolbach [12] observed that "in guinea pigs given an excessive amount of vitamin A (1000 to 1250 I. U. per gram weight) from the age of 10 days, the distal epiphysis of the femur and the proximal epiphysis of the tibia can be made to close in from 10 to 15 days—or at 20 to 25 days of age. These epiphyses normally close after 30 and 60 weeks of age respectively."

Ducks given from 1,000 to 1,800 units of vitamin A per gram of body weight per day for the first 13 days of life showed no abnormal features. These ducks were given 200,000 units of vitamin A per day for three weeks without showing any abnormal symptoms except a failure to gain as much in weight as the control birds and a decrease in the intensity of the color of their feet, legs and bills. This decrease in the amount of yellow pigment in the skin is interesting when the carotene content of the serum is followed. There was a marked decrease in the carotene content of the serum when excessive amounts of vitamin A were given to ducks on

11. Moore, T., and Wang, Y. L.: Hypervitaminosis A, Biochem. J. **39**:222, 1945.

12. Wolbach, S. B.: Vitamin A Deficiency and Excess in Relation to Skeletal Growth, Proc. Inst. Med. Chicago **16**:118, 1946.

a standard ration, as shown in Table 1. These chemical observations were made first and the variation in color of the skin was observed in a different group of ducks.

Wolbach [13] observed an ecceleration of bone growth in different species of experimental animals given large amounts of vitamin A. In fact, he stated that in rats and guinea pigs "it is possible to get the equivalent of a year's growth in a ten to fifteen day period." Nothing approaching that disturbance of growth has been observed in our ducks. Our observations show that the effects of hypovitaminosis and hypervitaminosis A are different in the ducks as compared with those reported by others in the rat.[11] The question, of course, arises as to why such variation occurs. This we cannot answer, but it is of interest to recall that the bones in the extremities of birds are different from those of mammals in that there are no independent epiphysial centers of ossification. In birds the ends of the bones remain cartilaginous to provide for growth in length.[14]

The question may be asked whether an adequate amount of vitamin A was given to our ducks to produce skeletal changes corresponding to those observed in mammals. This seems likely since we gave some young ducks 1,000 to 1,800 units of vitamin A per gram of body weight for 13 days, while Moore and Wang [11] gave rats, weighing 48 to 86 gm., 40,000 units of vitamin A acetate daily and found that "within 14 days all the rats in this group had fractures." We do not know what percentage of the vitamin A given to the ducks was absorbed from the gastrointestinal tract. However, the liver normally had an average of 7.3 γ of vitamin A per gram of fresh tissue, while those birds given 100,000 units of vitamin A had an average of 1,133 γ per gram of liver tissue. Furthermore, enough vitamin A was absorbed to affect the color of the skin.

The failure to demonstrate in the skeleton of the duck either pathologic or roentgenographic changes that might be attributed to vitamin A deficiency emphasizes the necessity of studying further the mechanism of the neurologic symptoms occurring in vitamin-A-deficient ducks. Such a study is now being made in our laboratory.

SUMMARY

The effects of hypovitaminosis and hypervitaminosis A have been studied in the duck. Birds failed to gain normally when fed a ration deficient in vitamin A and also when fed a standard ration supplemented with a large amount of vitamin A. Ducks fed the latter ration showed a diminution in the amount of pigment within the skin of their feet and legs. A decrease was found in the amount of carotene in the serum of ducks fed 100,000 units of vitamin A when they were compared with those fed 25,000 units of vitamin A. No significant pathologic or roentgenographic changes were observed in the bones of the extremities of the ducks fed either the vitamin-A-deficient rations supplemented with vitamin A or the standard ration supplemented with large amounts of vitamin A. From these experimental studies it is concluded that the effects of hypovitaminosis and hypervitaminosis A in the duck are different from those which have been described by others in the rat, the guinea pig and the dog.

13. Wolbach, S. B.: Pathology in Relation to Nutritional Research, Nutrition Rev. **3**:193, 1945.

14. Lillie, F. R.: The Development of the Chick, New York, Henry Holt & Company, 1908.

BONE-MARROW EMBOLISM

JOHN H. FISHER, M.D.
LONDON, ONT., CANADA

MY INTEREST in this condition was aroused following an autopsy on a young Indian man who suffered fractures of his fourth and fifth cervical vertebras and who survived five days. On examination of microscopic sections of his lungs bone-marrow emboli were found in the pulmonary arteries.

A review of the literature reveals few references to this condition. Brief mention was made by Henke and Lubarsch [1] of the fact that bone-marrow embolism may occur in eclampsia. MacCallum [2] merely stated that bone-marrow fragments may be introduced into the blood stream following mechanical injury to bones, such as fracture. Schenken and Coleman [3] reported one case of bone-marrow embolism associated with fat embolism following the operative reduction of a fracture of the neck of the femur. Lindsay and Moon [4] briefly reviewed, without comment, three cases of bone-marrow embolism following fractures and multiple other injuries.[5]

MATERIAL

Ninety-six consecutive unselected cases of bone fracture have been reviewed. Microscopic sections of the lungs were obtained at autopsy in a routine way, representing all lobes of both lungs, and were stained with hematoxylin and eosin.

OBSERVATIONS

In 19 of the 96 cases of fracture (19.8%) bone-marrow emboli were found in the pulmonary arteries. The emboli were never numerous. Sometimes only a single embolus was found in all the lung sections from a particular case. In other cases several emboli were present. Not infrequently the emboli were arrested at points of bifurcation of the pulmonary arteries and extended a short distance into the branches (Fig. 1). In all instances the bone-marrow emboli closely resembled normal bone marrow, being composed of fat and myeloid cells (Figs. *2* and *3*). Bone fragments were not encountered in the emboli.

Presented at the Forty-Eighth Annual Meeting of the American Association of Pathologists and Bacteriologists, Cleveland, April 26, 1951.

From the Department of Pathology, Faculty of Medicine, University of Western Ontario and the Meek Memorial Laboratory of Pathology, Victoria Hospital.

1. Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Springer-Verlag, 1931, Vol. 10, Pt. 3, p. 105-106.

2. MacCallum, W. G.: A Textbook of Pathology, ed. 7, Philadelphia and London, W. B. Saunders, 1940, p. 25.

3. Schenken, J. R., and Coleman, F. C.: Bone Marrow and Fat Embolism Following Fracture of Femur, Am. J. Surg. **61**:126, 1943.

4. Lindsay, S., and Moon, H. D.: Bone-Marrow Embolism Following Fracture, J. Bone & Joint Surg. **28**:377, 1946.

5. Since the preparation of this paper, an article entitled "Bone Marrow Embolism" by Henry Rappaport, Muriel Raum and Jonathan B. Horrell has been published (Am. J. Path. **27**:407 [May-June] 1951). The reader is referred to this excellent paper, which reviews the literature and reports *27* cases of this condition.

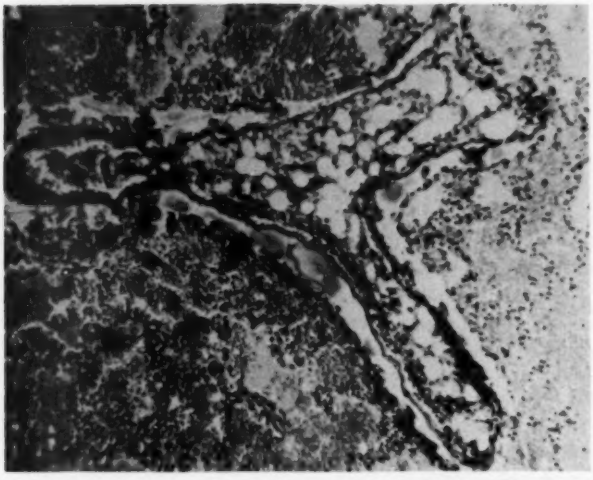

Fig. 1 (Case 11).—A bone-marrow embolus at the point of bifurcation of the pulmonary artery in the left lower lobe; × 120. The embolus completely fills the artery at this level.

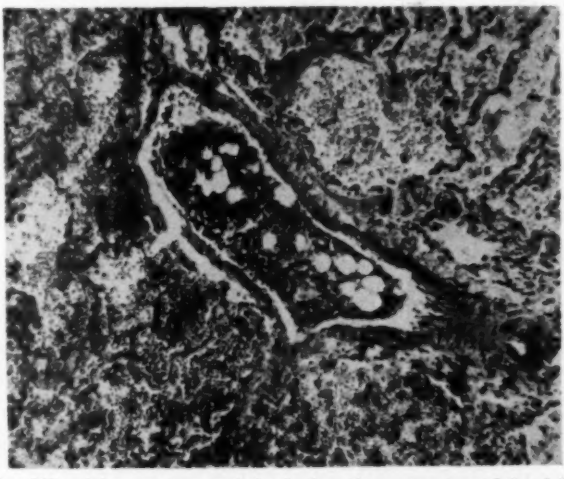

Fig. 2 (Case 11).—A bone-marrow embolus in the pulmonary artery of the right lower lobe; × 120.

The fractures resulted in most instances from automobile accidents. In all but one case, multiple fractures occurred. The bones involved in each case are designated in the accompanying

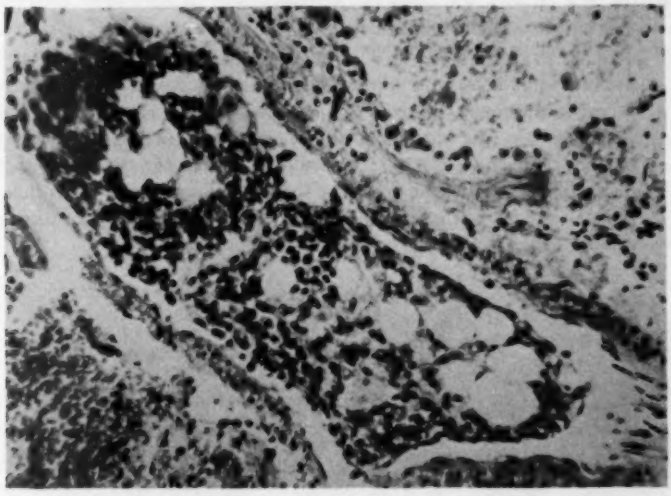

Fig. 3.—A portion of Figure 2, showing myeloid elements and fat cells; × 230.

Analysis of Nineteen Cases of Bone-Marrow Embolism of Lungs

Case	Age	Sex	Duration of Life After Injuries	Sites of Embolism in Lungs *	Fractures
1	82	M	2 hr.	R.L., L.U.	Tibia and fibula, radius and ulna, ribs
2	60	F	2 hr.	R.L., L.L.	Radius and ulna, femur, sternum, ribs, pelvis, vertebra
3	38	M	3½ hr.	R.L., L.U.	Radius and ulna, humerus, sternum, ribs
4	20	M	2 hr.	R.U., R.L.	Femur, ribs, pelvis
5	78	M	8 hr.	R.U. only	Skull only
6	70	F	About 1 hr.	R.M., R.L.	Humerus, femur, clavicle, sternum, ribs, skull
7	7	M	Instantaneous	R.U., R.M., L.U.	Tibia, fibula, ribs, vertebra
8	55	F	40 min.	All lobes, both lungs	Skull, ribs
9	88	F	Few min.	R.U. only	Skull, pelvis, tibia and fibula
10	39	M	6½ hr.	L.L. only	Vertebra, sternum, ribs
11	17	M	5 days	R.L., L.L.	4th, 5th cervical vertebras
12	65	M	½ hr.	R.U. only	Ribs, tibia, fibula, metacarpal, pelvis
13	69	M	¼ hr.	R.L., L.U.	Tibia, fibula, humerus, pelvis, ribs
14	67	F	8 hr.	R.L. only	Ribs, sternum, pelvis
15	86	M	12½ hr.	All lobes, both lungs	Ribs, pelvis, vertebras
16	57	M	6 hr.	R.U. only	Tibia, fibula, vertebra
17	52	F	Instantaneous	R.M., R.L.	Humerus, pelvis, ribs
18	33	M	6 days	R.L. only	Vertebrae only
19	45	M	20 hr.	R.U. only	Skull, ribs, clavicle

* R.L. means right lower lobe; L.U., left upper lobe; L.L., left lower lobe; R.U., right upper lobe; R.M., right middle lobe.

table. The duration of life after the injuries were inflicted varied from a few minutes (almost instantaneous death) to six days. The age and the sex of the patients and the location of the emboli in different parts of the lungs are given in the table. Bone-marrow emboli were not found in any other organ.

COMMENT

At the time that the lung sections were obtained bone-marrow embolism was not anticipated. The sections were taken in a routine way from all lobes of both lungs and must be considered as haphazard random samples.

The pulmonary arteries revealed a much higher incidence of bone-marrow emboli than anticipated before the survey was started; such embolism is much commoner than previously reported. It is my impression that if more numerous blocks of lung tissue or whole lobe sections had been examined, the incidence would have been still higher. Ninety-six cases of fractures are not a large series, but the study involved the examination of 480 microscopic sections of lung tissue and is thought to afford a reasonable index of the incidence of bone-marrow embolism of the lungs in fracture cases.

Fig. 4 (Case 7).—A bone-marrow embolus in the pulmonary artery of the right upper lobe of a child dying almost instantly after injury; × 230. This illustrates how rapidly bone-marrow embolism may occur.

In most of the 19 cases which were found in this survey the bone-marrow emboli were overlooked when the lung sections were first examined. They were found only after I became "bone marrow embolism conscious" and reexamined the microscopic sections of the lungs, looking specifically for bone-marrow emboli. The old aphorism "We see what we look for" is probably only too true. This fact may explain the low incidence of this condition previously reported. Either bone-marrow emboli have been seen and have been considered of no importance or they have been completely ignored.

Golodner, Morse, and Angrist [6] reviewed 304 cases of fracture of the hip. Eighty-six of the patients died, and, of these 86, 25 came to autopsy. In 9 of the 25

6. Golodner, H.; Morse, L. J., and Angrist, A.: Pulmonary Embolism in Fractures of Hip, Surgery **18**:418, 1945.

cases in which autopsy was performed pulmonary embolism was found. While the authors apparently were primarily interested in the finding of thrombi in the pulmonary arteries, they made no mention of bone-marrow emboli, and presumably none were seen. However, the interval between the occurrence of the fracture and the time of death varied from six to 70 days. The rather long interval might perhaps reduce the chance of finding bone-marrow emobli in the pulmonary arteries.

In the adult, beyond the age of about 25, under normal conditions, the bone marrow of the long bones becomes yellow and fatty, while that of the ribs, the sternum, and the vertebras remain red and cellular throughout life. Since in the cases here reported the fact that the bone-marrow emboli invariably were composed of fat and myeloid cells would suggest that they originated in red-marrow-containing bones.

In every instance the bone-marrow emboli appeared to be perfectly bland. No reaction was seen in the vessel wall at the point of arrest. No degenerative changes were observed in the marrow fragments. Thrombus had not formed about the emboli. They showed no endothelization or organization. However, it must be pointed out that in most instances the interval between injury and death was short.

In most cases the bone-marrow emboli occluded so few pulmonary arteries as to produce no detectable effects on pulmonary circulation. Pulmonary infarction was not observed. Fresh intra-alveolar hemorrhage was noted in many, but this finding was accounted for either by aspiration of blood or by direct trauma to the lungs and was considered to be unrelated to the emboli.

The rapidity with which bone-marrow embolism may occur is amazing (Fig. 4). In at least three cases (7, 9 and 17) death was almost instantaneous, and in several others the interval between injury and death was short.

In several instances bone-marrow embolism was associated with fat embolism. Since the surveyed material was not satisfactory for a study of fat embolism, I am unable to state accurately how often this association existed. However, it is certain that bone-marrow embolism can occur in the absence of fat embolism, and it is a common experience to find fat embolism without detectable bone-marrow embolism.

SUMMARY AND CONCLUSIONS

Bone-marrow embolism occurs much more frequently than has been previously reported. In a survey of 96 unselected cases of fractures, 19 cases (19.8%) of bone-marrow embolism of the pulmonary arteries were found. The emboli were few in any particular case and might have been easily overlooked. Apparently, they have been overlooked in the past. They obstructed so few pulmonary arteries that significant disturbances did not occur in the lungs.

Of the 19 patients, 13 were males and six females. Their ages varied from 7 to 86 years.

There was no predilection for any particular part of the lungs. However, in the surveyed material all the blocks of lung tissue included pleural surface and, therefore, were from peripheral parts of the lungs. In the various cases the right lower lobe, the right upper lobe, the left upper lobe, the left lower lobe and the right

middle lobe were involved in that order of frequency. The patients survived from a few minutes (almost instantaneous death) to six days after the injuries were inflicted. In one patient the skull alone was fractured, but in most of the patients multiple bones were fractured. Ribs, the pelvis, vertebras, the tibia and the fibula, the skull, and the sternum were the bones most frequently involved, in that order. In most cases the persons were subjected to very forceful violence in automobile accidents.

The bone-marrow emboli were bland and caused no reaction at the point of arrest. In some cases bone-marrow embolism was associated with fat embolism, but in others bone-marrow embolism occurred alone.

APPLICATION OF EMISSION SPECTROSCOPY TO MEDICAL PROBLEMS

I. Spectrographic Techniques

IVIE LEE SMITH, M.S.
ERNEST YEAGER, Ph.D.
NATHAN KAUFMAN, M.D.
FRANK HOVORKA, Ph.D.
AND
THOMAS D. KINNEY, M.D.
CLEVELAND

DURING recent years the techniques of quantitative emission spectroscopy have advanced to the degree that chemical and biological samples may be analyzed for metallic elements in a matter of minutes, with accuracies in some cases surpassing conventional chemical methods. Spectrographic techniques are particularly suited to medical problems because many metallic elements, particularly trace elements, may be readily determined simultaneously in complex systems where chemical methods are usually tedious and sometimes completely lacking. In addition, spectrographic analysis requires only small samples and is therefore particularly suited for the study of biological material.

Although biological systems were examined by spectroscopic methods as early as the 1920's, the majority of the applications reported in the literature has been restricted to the qualitative analysis or the semiquantitative analysis of a relatively few constituents at a time. The purpose of the present paper is to indicate the techniques which are most suitable for the quantitative analysis of biological specimens. In Part II [1] a review of typical applications of emission spectroscopy in the field of medicine will be presented. The bibliography will be included at the end of the second paper.[2]

The discussion which follows is intended to assist the medical worker in appreciating the relationship which spectroscopy bears to his own problems. A comprehensive treatment of each phase of emission spectroscopy is beyond the limits of this paper. The reader who is interested in applying spectrographic techniques in his work but has no previous experience in this field should consult one of the standard texts.[3]

This work is supported by a grant from the Damon Runyon Memorial Fund for Cancer Research.

From the Department of Pathology, Cleveland City Hospital, and the Departments of Chemistry and Pathology, Western Reserve University.

1. Smith, I. L.; Kaufman, N.; Yeager, E.; Hovorka, F., and Kinney, T. D.: Application of Emission Spectroscopy to Medical Problems: II. A Survey of Typical Applications, Arch. Path., this issue, p. 332.

2. If more than one article of an author is cited, the article is identified by the year of publication. If more than one article was published in the same year, identification is by year plus a letter, a, b, c, or d.

3. Ahrens. Brode. Harrison, Lord, and Loofbourow. Harvey. Nachtrieb.

The basic operations involved in using emission spectroscopy as an analytical tool are the excitation of the elements in the sample, the dispersion of the resulting spectrum into characteristic wave lengths, the recording of the spectrum, and the interpretation of the spectrum in a qualitative or a quantitative fashion. Of these operations, the excitation of the sample is the most critical as far as the success of the analysis is concerned. Each phase will be considered separately in relation to the over-all medical application of emission spectroscopy.

EXCITATION OF SAMPLE

Two steps are involved in producing characteristic radiations from a sample: volatilization and excitation. The method which is used to excite the sample is generally referred to as the source. Any source which is to be used for quantitative analysis must be reproducible with respect to both steps. The three types of spectrographic sources which have found most extensive use are the flame, the electric arc, and the high-voltage spark.

The flame, one of the first sources used in spectroscopy, is capable of exciting more than 34 elements with low ionization or excitation potentials. The material to be analyzed may be introduced into the flame by use of an atomizer for spraying solutions as in the Lundegårdh method [4] or fed into the flame as a solid or liquid sample dried on filter paper as in the method of Ramage.[5] The latter method was used by Ramage in conjunction with an oxycoal gas flame to detect more than 30 elements in human organs. The method has been extended to a quantitative basis by Stewart and Harrison, who used it to analyze potato extracts for lithium, potassium, rubidium, and cesium. The Lundegårdh flame method has been extensively investigated by Ells, who has established the optimum operation conditions. Generally, the errors in quantitative analysis with Ells's modifications of the atomizer technique are not larger than $\pm 5\%$ of the concentration of the element in the solution. In many cases the probable errors may be less than 1%. On occasion the reproducibility of the flame source is sufficient so that line intensities or their equivalent may be used directly as a measure of the concentration of the elements without recourse to the more complex internal standard method which is required for quantitative work with other sources. While the sensitivities are not as high as those attainable with the electric arc, they are adequate for many types of work.

Commercial flame photometers are available which use a modification of the Lundegårdh method in conjunction with a photoelectric recording system. These instruments have found extensive application in medical problems, particularly those involving body fluids, because of the ease with which they can be used and the relatively high accuracy.[6]

For many medical problems, flame methods are not adequate because of the restricted number of metallic elements which can be investigated and the relatively low sensitivities. The electric arc between conducting electrodes is a simple yet relatively inexpensive spectrographic source which does not have these disadvan-

4. Griggs, Johnstin, and Elledge. Lundegårdh, 1930, 1934a, 1934b, 1940.

5. Ramage, 1929, 1936. Fox and Ramage.

6. Domingo and Klyne. Dunker and Passow. Mosher, Boyle, and others. Natelson. Parks, Johnson, and Lykken. Proehl and Nelson. Robinson, Newman, and Schoeb. Schrenk and Smith.

tages. The popularity of this source for medical research is indicated by the fact that it is used in approximately 70% of the reported applications employing electrical excitation. Typical applications of the direct-current arc in the analysis of biological samples include the investigation of gallium in tissue (Bertrand) and trace elements in specimens such as cats' brains (Borovik), ductless glands (Borovik and Voiner), blood (Dutoit and Zbinder; Wolff, 1948a), bone (Kunowski), teeth (Lowater and Murray; Steadman, Hodge, and Horn), plant ash (Ewing, Wilson, and Hibbard; Rogers and Hughes), milk (Borovik and Kalinin), and skin (Schmid). In Figure 1*A* is shown the circuit involved in the direct-current arc.

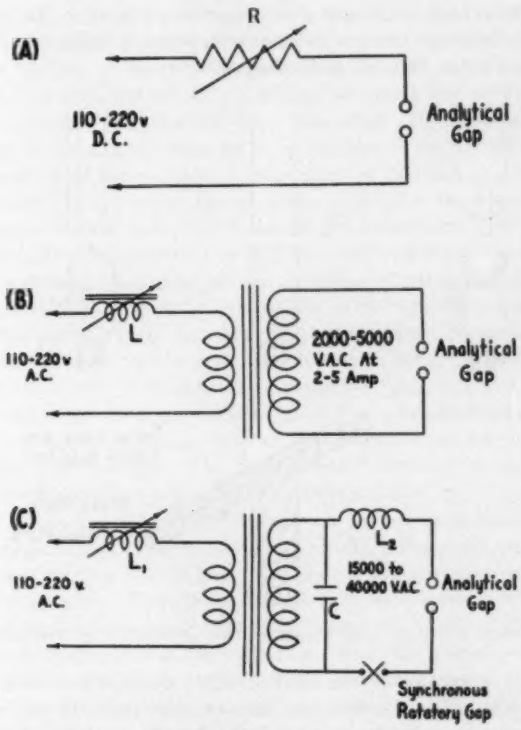

Fig. 1.—Source units.

This source is characterized by relatively high average temperatures in the gap (3,000-6,000 C.) as well as high electrode temperatures. Between 3 and 15 amp. are used in analytical work with a voltage drop of approximately 50 volts across the analytical gap and the remainder across the variable ballast resistance (*R*). Generally, the sample electrode is made the anode, since this is the hotter electrode. While the sensitivity of the arc may be extended to as low as 0.01 μ g. without any special effort, the reproducibility is poor, primarily because of the lack of precision in the volatilization step. Selective distillation of the sample takes place

in an erratic fashion, in part because of the tendency of the arc to burn between a few very restricted spots on the electrodes. In quantitative work with the direct-current arc the total-combustion method is often used to minimize these effects. In this technique the sample is completely volatilized, and hence the effects of selective distillation should cancel out.

Graphite electrodes are used with the direct-current arc in preference to other conducting electrodes because higher electrode temperatures are reached. Copper (Lindemann), silver, or aluminum electrodes may be substituted at some sacrifice of sensitivity when it is desirable to eliminate the cyanogen bands which often interfere with the lines of some of the elements under investigation. Nitrogen-free atmospheres have also been used for the same purpose, e. g., carbon dioxide (Johnson and Norman), oxygen (Steadman), steam (Smith and Wiggins), and the rare gases (Vallee, Reimer, and Loofbourow).

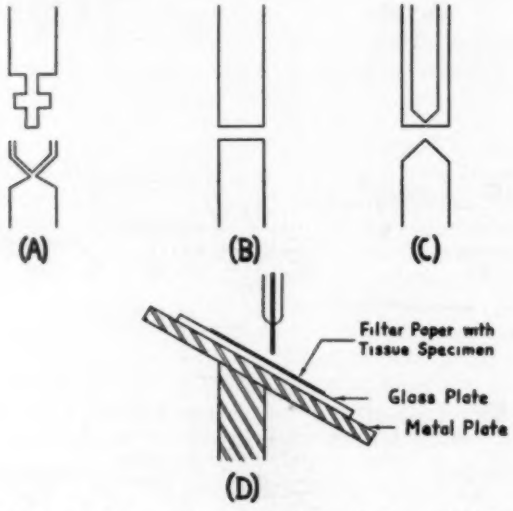

Fig. 2.—Typical electrodes used in spectroscopic examination of biological material.

On occasion, impurities in the graphite electrodes which do not appear in the blank run because of low volatility may become sufficiently volatile in the presence of the sample, e. g., chlorides, to interfere with the analysis. For this reason, spectrographic "high purity" graphite is recommended for all medical work involving trace elements. Preshaped electrodes can be secured commercially for use in the direct-current arc. Many forms [7] of electrodes have been devised for handling samples in the arc. Sensitivity and reproducibility are very dependent on electrode construction. For fresh, dried, or ashed specimens, a graphite lower electrode of the type shown in Figure 2*A* is a convenient means for supporting the sample. In quantitative analysis the arc is continued until the lower electrode is

7. C. E. Harvey presents a discussion of various types of electrodes for the arc in his text "Spectrochemical Procedures," pp. 335-348.

burned to a notch just below the cup in order that a constant amount of graphite as well as the complete sample will be volatilized each time. The notch on the upper electrode is to minimize the tendency of the arc to wander up the side of the upper electrode. Microversions of these electrodes may be used for the analysis of small samples. Solutions may be dried on the end of a plane lower electrode (Fig. 2*B*) or evaporated to dryness in a cupped electrode (Fig. 2*A*). In general, probable errors of at least 20% are to be expected in using such techniques with the direct-current arc.

The high-voltage alternating-current arc has also been used as source [8] in biological studies.[9] A simplified circuit diagram is given in Figure 1*B*. Operation is usually with 2,000 to 5,000 volts at 2 to 5 amp. in the secondary circuit. The alternating-current arc is more stable than the direct-current arc and considerably more reproducible. Since the arc is interrupted and established 120 times per second (for 60-cycle line frequency), the problem of arcing between a few restricted points on the electrode surfaces is, for the most part, eliminated. In quantitative work with the alternating-current arc, the material to be analyzed is usually placed in solution and a fixed amount of the solution is evaporated to dryness on the top of a flat graphite electrode. On occasion the probable error may be as small as 5%, a considerable improvement over direct-current arc techniques. The sensitivity of the alternating-current arc, however, is less than with the direct-current source.

A spark is distinguished from an arc in terms of the instantaneous and the average current (Williams and Scott). While in the arc the current and hence the temperature are relatively constant, the instantaneous currents in the spark source may amount to thousands of amperes for microsecond periods. The average current, however, is low. Although the instantaneous gap temperatures may be as high as 10,000 C., the average temperature of the solid electrodes is low. The sensitivity of the spark source is very limited unless the sample can be injected directly into the analytical gap. The spark source,[10] however, is capable of a precision not normally attained with the direct-current arc.

In Figure 1*C* is a simplified circuit diagram for a typical spark source. The transformer should supply between 15,000 and 40,000 volts in the secondary circuit. The synchronous rotating control gap (A_c) is used to control the voltage at which the discharge takes place. Comparable results have been obtained with an air-blast gap in place of the synchronous rotating gap (Enns and Wolfe). By changing the condenser and the inductance in the secondary circuit, the discharge may be varied from the spark-like extreme to a more arc-like character.

Nonconducting samples, such as ashed specimens, usually cannot be handled directly in the spark with good results. Two quantitative techniques have been developed in recent years which offer promise in the medical field. In the copper-spark method (Fred, Nachtrieb, and Tomkins) the sample to be analyzed is converted to the chloride and the resulting solution dried on two flat-copper electrodes (Fig. 2*B*). These two electrodes are subsequently sparked against each other under controlled conditions. The sensitivities obtained with this technique are in many cases beyond those generally available with the direct-current arc, and the repro-

8. Duffendack and Wolfe.
9. Hess, Owens, and Reinhardt. Owens. Patterson.
10. Duffendack, Wiley, and Owens. Lamb.

ducibility is between 5 and 10%. "Volatilization" is accomplished by literally "blasting" the sample off the surface of the electrode with the spark. The copper-spark method is subject to extraneous-salt effects; i. e., a large concentration of one component may influence the intensity-concentration relationships for another component. By introducing a fixed amount of a given salt in each run, it is possible to lower the concentration of all other materials to the point where the "environment" in the discharge is relatively constant. While the sensitivity is reduced by this procedure, it is still more than adequate for most trace analysis. One limitation of the copper-spark method is that only chlorides are suitable. Other acid solutions will either attack the copper directly or require such high temperatures in evaporating them to dryness that the copper will oxidize appreciably. Other pure metals with simple spectrums can be used to replace the copper electrodes.

The porous-cup method developed by Feldman is designed to permit the direct analysis of solutions with the high-voltage-spark source. Although other anions may be used, the sample is generally converted to sulfates and the solution placed inside a hollow graphite electrode of the type shown in Figure 2*C*. The solution electrode is then presparked through the bottom for a fixed time in order to make the bottom of the carbon electrode porous to the solution. After a fixed delay, i. e., 15 sec. following the presparking, the upper electrode is sparked and the spectrum recorded. The sensitivity of this method approaches that of the arc, and the reproducibility is of the order of 5 to 10%. In the porous-cup method, however, a change in concentration of one solute present in large amounts may shift the analytical curves relating line intensities to composition for other constituents. The most satisfactory method of minimizing this effect is to use standard solutions which resemble the sample solution as closely as possible. The porous-cup method has been recently applied by us [11] to the quantitative analysis of a number of metallic elements in human tissue. In this work the sensitivity of the porous-cup method, as well as the quantitative precision at low concentrations, has been extended for many elements through the use of a helium atmosphere for the suppression of background.

The high-frequency-spark technique of Gerlach and Gerlach [12] is useful for the qualitative and perhaps semiquantitative analysis of untreated tissue sections. The specimen to be analyzed is placed on a piece of moistened filter paper, which in turn is placed on a thin glass plate. The latter is mounted on a metal plate as shown in Figure 2*D*. The upper electrode is either a gold or a platinum wire. The high-frequency uncondensed spark is usually obtained from a Tesla coil. Electrical contact is effectively made to the specimen through the alternating-current capacity between the metal holder and the moistened filter paper. In operation the sample and filter paper are continuously moistened with a sodium nitrate solution to prevent the specimen from flaming. The lower holder is moved with the top electrode fixed in position; thus, specific parts of the specimen may be examined. This technique has sensitivities approaching that of the arc but is not designed for quantitative work.

At the present time the flame source (Lundegårdh), the porous-cup method, and the copper-spark method seem most promising for medical applications where

11. Yeager, Smith, Kaufman, Hovorka, and Kinney.

12. Benoit. Berton, 1947, 1949. Gerlach and Garlach, 1934b. Morel and Policard. Policard, 1933, 1935. Policard and Morel, 1932a. Schwarzacher.

quantitative results are required. In the past, however, a source technique, previously of limited reproducibility, has been converted into a quantitative source through some minor variation. The "frontier" of analytical emission spectroscopy is the development of improved excitation techniques.

The sensitivities of the various methods of excitation are compared in Table 1. The values in this table are intended to indicate the general sensitivities for each source but are not absolute in any sense inasmuch as the limits of detection are dependent on specific factors, such as the exact source conditions, the presence of interfering elements, the dispersion and speed of the spectrograph and the method of recording the spectrums.

TABLE 1.—*Limits of Detection in Millimicrograms with Various Types of Excitation*

Element	D.C. Arc*	Copper Spark†	Porous Cup‡	A.C. Arc§	Flame‖
Ag	10	...	300	..	5,000
Al	20	10	300	60	.....
As	1,000	500	30,000	..	.....
Ba	...	10	15,000	..	.....
Be	...	0.2	8	..	.....
Bi	20	20	1,500	..	.....
Ca	...	10	.....	60	1,000
Cd	400	200	30,000	..	.....
Co	...	50	700	..	30,000
Cr	200	5	700	10¶	.....
Cu	20	...	300	60	3,000
Fe	20	50	800	60	30,000
Hg	200	500	1,500	..	.....
K	...	10	70,000‖	..	20,000
Li	...	0.2	30	..	.....
Mg	10	1	3	60	10,000
Mn	20	2	700	60	1,500
Na	2,000	10	10,000‖	..	25,000
Ni	400	10	3,000	60	30,000
P	5,000	2,000	25,000	..	.....
Pb	20	5	3,000	60	.....
Sb	400	500	30,000	..	.....
Si	200	10	.....	250¶	.....
Sn	20	...	30,000	60	.....
Sr	...	50	150‖	..	.....
Tl	...	10	1,000	..	.....
U	...	100	.....	..	.....
Zn	2,000	50	8,000	300	.....

* Pierce, Torres, and Marshall.
† Fred, Nachtrieb, and Tomkins.
‡ Feldman. The values are on the basis of a 0.3-cc. sample.
§ Hess, Owens, and Reinhardt.
‖ Griggs, Johnstin, and Elledge. The values are on the basis of a 2-cc. sample.
¶ Duffendack and Wolfe.
‖ The value does not represent the ultimate sensitivity.

THE SPECTROGRAPH

Spectrographs which are to be used for trace analysis should have a high linear dispersion. The ultimate sensitivity of any excitation technique is set by the point at which the characteristic lines can no longer be distinguished from the background. With increasing dispersion, the background is spread over a larger area on the photographic plate and hence diluted, while the line width is essentially independent of the dispersion, provided the resolving power and slit width remain constant. For a given spectral line the sensitivity may be expected to increase directly as the linear dispersion. Large linear dispersion and high resolving power are especially

necessary in medical work, since the spectrums of specimens containing iron are rather complex. In addition, the instrument should be suitable for work with the visible as well as with the ultraviolet spectral regions. The photographic plate or the film holder should be sufficiently long to permit a reasonable region of the spectrum to be included in one exposure, since the sample available on some occasions may be sufficient for only one burn. High speed is also useful when the quantity of sample is very limited; however, this is usually found only with instruments of low linear dispersion.

Both grating and prism spectrographs are commercially available which are capable of being satisfactorily applied in biological analysis in terms of the above criteria.[13] For a given cost, the grating instrument in general seems superior to the prism instrument in performance. The optical properties of each are compared in Table 2.

TABLE 2.—*A Comparison of Grating and Prism Spectrographs*

	Grating	Prism
1. Linear dispersion	Constant *	Varies as a function of wave length
2. Resolving power	Constant *	Varies as a function of wave length
3. Extraneous lines	Lyman and Rowland ghosts; overlapping diffraction orders	Absent
4. Speed	Essentially comparable	Essentially comparable
5. Spectral range	Single set of optics covers ultraviolet and visible regions	Dual optics (quartz and glass) necessary to cover effectively ultraviolet and visible regions
6. Minimum cost of instrument satisfactory for general medical research	$4,500	$6,500

* Provided the camera is located on the normal to the grating, as is the case with most commercial instruments.

RECORDING OF THE SPECTRUM

For the quantitative analysis of medical samples for major, minor, and trace elements simultaneously, the photographic emulsion should have the following characteristics:

1. Medium contrast (2 to 3 γ)
2. Relatively high speed
3. Constant speed and contrast throughout the range of wave lengths involved in the analysis
4. Fine grain and high resolving power

In practice, the contrast of an emulsion is related to the speed of the emulsion: photographic plates with high speed usually have low contrast and vice versa. Whenever a sufficient sample is available, however, speed is secondary to contrast requirements. In qualitative work with trace elements, high contrast is desirable in order that faint lines may be distinguished from the background. When a large range of concentrations is involved in quantitative work, a lower contrast is more satisfactory.

Unfortunately, the majority of the photographic emulsions available for spectrographic analysis exhibit considerable variation in contrast and speed with wave

13. Boyle and others. Foster, 1936. Kreider. Wilson.

length. Thus the characteristic curves [14] relating photographic density to light intensity must be determined for the emulsion for each section of the spectrum involved in the quantitative analysis.

In qualitative analysis, exact control of the photographic processing is not necessary. In quantitative analysis, however, parameters such as the development temperature, the condition of the developer, the time of development, and the amount of agitation must be kept constant to minimize photometric errors in the determination of line intensities. For a given type of emulsion there may still be sufficient variation between individual plates to require a determination of the characteristic curves of the emulsion for each plate. An exact photographic routine will extend the accuracy in quantitative analysis.

INTERPRETATION OF SPECTRUM

Quantitative results are usually desired in the analysis of medical specimens. Qualitative analysis, however, is a preliminary step to quantitative work.

All of the elements may be identified from the spectrums under proper excitation conditions, but only approximately 75 elements, essentially metallic, are detectable with the usual arc or spark source. The qualitative analysis of the majority of these elements with emission techniques is a relatively simple process. After the sample has been excited (usually with the arc), the individual spectral lines may be identified by comparing them with the spectrums from known samples or by measuring wave lengths and comparing them with a table of wave lengths. In conjunction with the former technique the *Raies Ultimes* powder available through Adam Hilger (London) is very useful. In the arc this material will produce at least seven lines for each of 50 of the most important elements. The measurement of wave length for identification purposes may be accomplished by placing the spectrogram of a reference element such as iron, with many lines of known wave length, adjacent to the unknown spectrogram. In addition to these techniques for associating the spectral lines with particular elements, distinct spectral patterns characteristic of given elements may be recognized, i. e., the quintet of the magnesium spectrum, the doublets of the aluminum spectrum.

The "Atlas der Restlinien" of Gatterer and Junkes is particularly useful in the identification of spectrographic lines. This work, published in three volumes, contains tables and reproductions of the arc and the spark spectrums of 73 elements, including some metalloids (chlorine, bromine, iodine, sulfur, selenium).

For the qualitative analysis of trace elements, the lines with the maximum absolute sensitivity, i. e., the persistent lines, are sought for identification purposes. No definite minimum-concentration limits can be assigned to the detection of the elements, for sensitivity is very dependent on factors ranging from the type of source through the nature of the photographic emulsion. The lower limit for the "easier" elements is of the order 10^{-8} gm. In detecting a trace element in a complex mixture the investigator must carefully consider interference from other constituents as well as from contamination before concluding that the identification is

14. For details of the procedure for preparing the characteristic curves for a photographic emulsion, the reader should consult one of the reference texts in the bibliography (Ahrens; Brode; Harrison, Lord, and Loofbourow; Harvey; Nachtrieb). A description of the various types of plates and films available for spectrographic analysis is given in the Eastman Kodak booklet "Materials for Spectrum Analysis."

positive. When the persistent lines of a trace element are almost entirely "covered" by lines of more prominent elements, it may be necessary to use techniques such as noting the change of line intensity with excitation conditions or extracting the trace element or the interfering elements chemically. These are not standard procedures but may be necessary when the positive identification of a trace element is critical.

Quantitative emission spectroscopy is based on the observation that line intensities vary with concentration. Since a number of factors contribute to spectral intensity, extensive precautions must be taken to insure that concentration is the only effective variable. With the exception of the flame source, it is not possible to eliminate appreciable variations in source conditions between determinations. The method of internal standards is used to prevent such variations from seriously limiting the accuracy of the analytical procedure. With this method the ratio of the intensity of a line characteristic of the particular element to the intensity of a control line is correlated with the concentration of the element. The control or reference line must be produced by an element of fixed concentration which is either added to the sample or occurs naturally in the sample. The two lines should have wave lengths as well as photographic densities which are close in value and the excited electronic-energy states producing both lines should be similar in order that variations in source conditions will effect each in the same fashion. In multielement quantitative analysis, a transitional element, such as molybdenum, is often added as the reference element because it provides a large number of lines throughout the spectrum from which to choose control lines.

Analytical curves relating the line-intensity ratios to the composition are prepared from a series of standard samples containing known amounts of the elements involved in the analysis, together with the internal standard elements in fixed concentrations. For medical work, solution techniques and hence standard solutions seem the most satisfactory in terms of convenience and accuracy. The standard samples should have a composition as close as possible to those of the unknown samples. Often the standards as well as the unknowns are diluted with a buffer agent, such as a salt, to prevent the analytical curves for one element from being shifted by a change in amount of another major component in the system.

After the analytical curves have been prepared for each element in question, the composition of an unknown sample may be determined from the line-intensity ratios by consulting the analytical curves. The line intensities are usually determined from densimetric measurement on the spectrograms and the characteristic curves for the emulsion.

Many abbreviated forms of the above treatment have been devised. They are usually not satisfactory in the quantitative application of emission spectroscopy to medicine, primarily because the samples are heterogeneous.

Spectral-line width has also been used by Eastmond and Williams as an indication of concentration in the analysis of zinc in biological ash with the direct-current arc.

PREPARATION OF BIOLOGICAL MATERIAL FOR ANALYSIS

All biological material must be pretreated prior to quantitative spectroscopic analysis. In many cases the samples are dehydrated to a constant weight by means of a vacuum drying oven, vacuum desiccation over concentrated sulfuric acid, or

similar procedures. The investigator then has the option of expressing the quantitative results, subsequently, in terms of dry weight, as well as in terms of original weight.

In qualitative analysis with the arc, the organic matter should be removed by ashing before the sample is excited, in order to obtain maximum sensitivity. Either dry or wet oxidation may be used to remove the organic constituents. With the former, the oxidation may be carried out in platinum or fused quartz at temperatures approaching 500 C. The investigator must establish, however, that volatile components of interest in the analysis are not lost. Dry oxidation is generally preferred for qualitative analysis of trace elements because the mineral constituents are concentrated with a minimum possibility of contamination. Inasmuch as tissues are usually placed in solution prior to quantitative analysis, wet oxidation is often more convenient. The dehydrated, pulverized specimen can be oxidized with small volumes of hot concentrated mineral acids. Sulfuric, nitric, or perchloric acid, or mixtures of these will effectively destroy the organic portion of animal tissue.

If the solutions are to be used with the copper-spark technique, the sample should be converted to chlorides; sulfates and nitrates should be avoided. Chlorides usually give the best sensitivities with the Lundegårdh flame method, also. In the porous-cup-electrode technique the sulfates appear to be the most satisfactory, although other anions can be used.

When trace elements are involved, chemical methods can greatly extend the range of spectrographic analysis. Electrodeposition (Urbain), precipitation techniques (Mitchell and Scott), carrier precipitation (Mitchell and Scott), solvent extraction (Nachtrieb and Conway), and selective adsorption can be used in quantitative work to concentrate trace elements as well as to reduce the concentration of interfering elements. For example, iron, which often interferes in the identification of trace elements because of its rich spectrum, can be effectively removed by extraction of the chloride from acid solution with ether (Nachtrieb and Conway). Precautions must be taken, however, lest the sample be contaminated by the chemical reagents, and blank determinations should be made.

APPLICATION OF EMISSION SPECTROSCOPY TO MEDICAL PROBLEMS

II. A Survey of Typical Applications

IVIE LEE SMITH, M.S.
NATHAN KAUFMAN, M.D.
ERNEST YEAGER, Ph.D.
FRANK HOVORKA, Ph.D.
AND
THOMAS D. KINNEY, M.D.
CLEVELAND

IN A PREVIOUS paper [1] the techniques of emission spectroscopy were discussed as employed in the analysis of biological material. The present paper deals with these techniques as applied to the solution of problems in medicine, pharmacology, and pathology.

The majority of the medical applications of emission spectroscopy reported in the literature have been concerned with (1) the determination of the mineral elements present in normal human organs and body fluids, (2) the assimilation and elimination of metallic elements administered for therapeutic purposes, and (3) qualitative and quantitative variations of metallic elements in pathological conditions.

ANALYSIS OF NORMAL HUMAN ORGANS AND BODY FLUIDS

In 1933 Boyd and De analyzed qualitatively a number of human organs with the direct-current arc. Identification of specific elements was accomplished by comparing them with spectrograms obtained with *Raies Ultimes* powder. In addition to the usual alkali-metal and alkali-earth elements, these workers found that manganese is widespread in distribution and is found especially in the pancreas, the kidney, and the liver; that zinc, aluminum, and vanadium are present in the kidney and the liver; that lead and tin are found in a number of organs, although not in all organs, and that iron and copper are present in all organs.

In 1936 Scott and McMillen investigated human spinal fluid. The dry residues from 2-cc. samples were burned on graphite electrodes in the direct-current arc. Sodium, calcium, magnesium, copper, potassium, phosphorus, barium, strontium, and boron were found in all samples, while most samples contained lead and aluminum. Tin was occasionally found.

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From the Department of Pathology, Cleveland City Hospital, and the Departments of Chemistry and Pathology, Western Reserve University.

1. Smith, I. L.; Yeager, E.; Kaufman, N.; Hovorka, F., and Kinney, T. D.: Application of Emission Spectroscopy to Medical Problems: I. Spectrographic Techniques, Arch. Path., this issue, p. 321.

Lee and Thomson and Lee applied a quantitative spectrographic method to the analysis of urine and saliva that proved satisfactory in connection with metabolism experiments. The method of excitation involved introducing a solution directly into a high-voltage spark gap. Results for sodium and potassium were reported with an average error of less than 3% of the amount present, and slightly less accuracy was noted for calcium and magnesium. The typical analytical results obtained by the spectrographic method are shown in Table 1. Duffendack, Thomson, Lee, and Koppius also described a method for determining small quantities of sodium, potassium, calcium, magnesium, and lead in biological materials.

Cholak and Story (1938, 1941) [2] and Kehoe, Cholak, and Story (1940a, 1940b) studied extensively the normal range of concentrations of manganese, lead, tin, aluminum, copper, and silver in human organs and body fluids. The quantitative procedure consisted of acid oxidation, evaporation to dryness, solution in a buffer salt base, and subsequent reevaporation on graphite electrodes. The direct-current arc was used for excitation. Below is a brief summary of the normal occurrence of these metals as determined in biological material in the studies cited.

Manganese: Average concentration in most organs, 0.03-0.04 mg./100 gm. of fresh tissue; regularly found in the blood of normal animals in concentrations of about 0.015 mg./100 ml.; concentration in urine, less than 0.01 mg./l.

TABLE 1.—*Quantitative Analysis of Urine and Saliva*

	Saliva, Mg./100 Cc.	Urine, Mg./100 Cc.		Saliva, Mg./100 Cc.	Urine, Mg./100 Cc.
Mg	0.15	10	K	88.0	210
Na	30.0	350	Ca	5.3	12

Copper: Blood concentrations, 0.09-0.11 mg./100 ml. (concentration not constant); liver, 0.30-0.91 mg./100 gm.; brain, 0.36-0.68 mg./gm.; bone, 0.37-0.47 mg./100 gm.

Aluminum: Ingestion of 200 mg./day harmless; regularly present in human tissues in small amounts; mean concentration in blood is 0.013 mg./100 ml.

Tin: Found irregularly in normal biological tissue, or about 80% of samples.

Silver: 10% of human tissue samples contain silver; concentration, less than 0.01 mg./100 gm.

Lead: Found in skeleton (amounts do not correlate with age); normal blood contains 0.01-0.06 mg./100 gm.; urine, about 0.03 mg./l.

Cholak, Hubbard, and Story (1943) published a direct-current arc spectrographic method for determining aluminum in biological material in the range of 1 γ to several hundred milligrams per sample. They reported the urinary concentration of aluminum as 0.04-0.06 mg./l., and the blood content as 0.03 mg./100 gm.

Cholak and Hubbard (1944b) have also reported a method, using the Lundegårdh atomizer and an air-acetylene burner, applicable to the determination of magnesium, sodium, iron, potassium, and calcium in 2 ml. of whole blood; magnesium, sodium potassium, and calcium in 2 ml. of serum or plasma; and magnesium, sodium, potassium, calcium, iron, and copper in 5 gm. of tissue samples.

2. When more than one article of an author is cited, each article is identified by the year of publication. If more than one article was published in the same year, identification is by year plus a letter, a, b, c, or d.

The major limitation of the technique of these workers was the relatively large amount of sample required for the analysis, particularly tissue samples. Typical analyses with the air-acetylene flame are reported in Table 2.

Hald has discussed the advantages and limitations associated with the flame photometer for analysis of sodium and potassium in biological material. A detailed description was given of the preparation of material for analysis and the optimum concentrations for the measurement of sodium and potassium in serum, whole blood, food, feces, and urine.

Overman and Davis used the Perkin-Elmer flame photometer for sodium and potassium determinations on blood, plasma, red blood cells, and urine. Average flame photometer data for sodium and potassium analyses on 30 human subjects are given in Table 3.

Robinson, Newman, and Schoeb, in a recent publication, described a method for using the flame source to obtain quantitative values for sodium, potassium, magnesium, calcium, silver, copper, iron, strontium, and lithium in human and cow's milk, blood, urine, feces, and tissue with an accuracy of 5%.

TABLE 2.—*Quantitative Analysis Using Flame Photometer (Cholak and Hubbard, 1944b)*

	Mg	Na	Fe	Mn	K	Ca
Urine (human), gm./1,000 ml.....	0.100	3.90	.....	.....	2.20	0.035
Liver, gm./100 gm.....	0.0166	0.120	0.0006	0.0002	0.250	0.0073
Brain, gm./100 gm.....	0.0180	0.120	0.0052	.....	0.380	0.0073

TABLE 3.—*Quantitative Analysis Using Flame Photometer (Overman and Davis)*

	Whole Blood	Plasma	Red Blood Cells	Urine
Na, (mg./100 cc.).....	218	352	14	220
K, (mg./100 cc.).....	222	20	437	197

In addition to supplying information as to which mineral elements are present in human organs,[3] spectrographic techniques also have aided the physiologist in establishing the source and the role of these elements in body functions.[4] A few examples will be cited.

Voinar and Rusanov have applied spectrographic techniques to the chemical topography of the metallic elements in the human brain. According to their work, those sections of the brain with different morphological structures and physiological functions differ in composition with respect to the trace elements aluminum, chromium, manganese, molybdenum, nickel, lead, silicon, tin, titanium, vanadium, zinc, silver, and copper.

During the early use of aluminum kitchenware, many conflicting reports appeared on the assimilation of the metal and its role in physiology. McCollum, Rask, and Becker in 1928 published results of spectrographic studies in which they

3. Cholak and Hubbard, 1944a. Cohn. Foster and Horton. McHargue, Offutt, and Hodgkiss. Mankin. Neveu. Norinder (1938, 1940, 1942). Pfeilsticker. Ruthardt. R. G. Smith and others. Steadman (1941). Stecher, Bedell, and Levis. Weber and Rulkavina. Weil. Wolff, 1948b, 1950.

4. Daniel, Hewston, and Kies. Fox and Baer. Garde. Hoagland. Hoagland and Stone.

reported that aluminum is not a constituent of plant or of animal matter, is not absorbed from the diet, and exerts no deleterious action. Kahlenberg and Closs in 1929 repudiated the previous results by analyzing a large number of samples, including human breast cancer. The dry ash was arced on copper electrodes and aluminum was found to be present in all samples. Lewis in 1931 used the spectrograph to study the assimilation of aluminum in blood and milk. The specimen was ashed in quartz and a 10-mg. sample burned in a direct-current arc. It was noted that normal blood does not contain aluminum and that the metal is found in both blood and milk after feeding just one meal of aluminum-containing food. Kehoe, Cholak, and Story (1940b) reported that aluminum was found in all samples of human tissue and that the ingestion of 200 mg. of the metal per day is harmless.

Drea in 1936 determined the trace elements in dental tissue by exciting dry-ashed samples in the direct-current arc. With the source, fluorine can be detected in terms of the band spectrum of calcium fluoride (5290 A.) provided calcium is also present. Drea demonstrated by the semiquantitative analysis of 18 geographically chosen specimens that fluorine is stored in the teeth of people living in areas where the element is present in the drinking water.

In 1937 White, Bunting, Thomson, and Jay attempted to correlate the condition of the teeth with the mineral composition of the saliva. They found that high potassium content (above 50 mg./100 cc. of solution) was associated with caries; that low potassium content (below 35 mg./100 cc. of solution) was associated with freedom from caries and that the sodium content of the saliva showed no definite relationship with the presence or the absence of dental caries.

Foster, Langstroth, and McRae in 1938 measured the concentration of sodium and potassium in unspecified glandular secretions with the high-voltage alternating-current condensed-spark source and the internal standard method. These investigators stated that quantitative data of this type should pave the way to an understanding of the behavior of glands.

Kehoe, Cholak, and Story (1940a) determined the trace elements in successive samples of food and feces with the same spectrographic techniques as were used in their general investigation of human organs described previously. For the most part, they found that the amounts of the trace elements retained by the body were very small compared with the amounts eliminated.

In a comprehensive study of mineral metabolism in childhood, Brody and Ewing have used the direct-current arc for the analysis of samples of food and feces. The samples were obtained from eight children during 11 consecutive five-day periods. Each sample represented a composite for five days. Results are in agreement with the conclusions of Kehoe, Cholak, and Story cited above and have been evaluated in a separate publication (Macy).

Some investigators, particularly those in the 1930's, felt that a comparison of the arc and spark spectral intensities might give an indication of the binding strength for a metal in complex organic molecules. Policard and Morel (1932b) examined blood with both arc and spark excitation and found that iron was not apparent in the spark spectrum, although it was very evident in the arc spectrum. They interpreted these results as indicating a rather rigid combination of iron in hemoglobin. Such an interpretation of the relative intensities in the arc and spark spectrums is generally not justified. The sensitivities of both sources are related to the volatility of the compound and excitation conditions. Once the compound is vaporized in

the analytical gap, the dissociation of organometallic compounds shall take place very readily. Evidence of this can be found in the general lack of band spectrums of metal-carbon fragments in either source.

APPLICATIONS IN THE FIELD OF THERAPEUTICS

When metallic compounds are used as therapeutic agents, it is desirable to know how the metallic elements are assimilated, stored, or eliminated by the human body. Such information is particularly important in the use of heavy metals, where rigid control of dosage must be exercised to minimize toxic effects.

In 1925 Bayle, Fabre, and George followed the elimination of bismuth in the urine after the salts had been administered in therapy of syphilis. In this investigation a 24-hour specimen was evaporated to dryness and ignited to a white ash. The residue was then dissolved in hydrochloric acid and the bismuth concentrated by precipitation with hydrogen sulfide. The precipitate was excited with the high-voltage spark. Bismuth was generally detected spectrographically in the urine between three and five days after treatment. The physical activity of the patient affected the time of appearance; e. g., with bed patients, bismuth is detected in the urine later than in cases in which the patients are mobile.

Pollicard and Boucomont reported a case of a 4-yr.-old child suffering from kala-azar. The child had received antimony salts equivalent to about 2 gm. of the metal over a period of seven months. The histospectrographic procedures of the Gerlachs (1934b) revealed the presence of antimony in the lung, the liver and the spleen. The metal was not found in the blood, the walls of the pulmonary artery, or the bronchial walls.

According to Gaul and Staud (1935a), the spectroscopic analysis of connective tissue for metallic constituents is more significant clinically than the usual analysis of excretory products for toxic metals. These investigators have employed spectrographic techniques to collect data on the extent to which gold (1933) and silver (1934a, 1935d) are retained in the skin during therapy with the corresponding salts. Skin samples weighing approximately 0.025 gm. were removed with a 0.47-cm. punch and placed in the crater of a graphite electrode for analysis with the direct-current arc. The spectrograms of standard samples were also included on each photographic plate for purposes of estimating the order of magnitude of the concentration of the silver or the gold in the skin samples.

Gaul and Staud (1934a) applied this procedure in studying a number of cases of argyria caused by silver arsphenamine used in the treatment of syphilis. First, skin specimens from untreated individuals were examined to establish the normal occurrence of silver; then 13 biopsy specimens from patients who received known doses were analyzed. Silver was found to be uniformly distributed throughout the cutis. The concentration of silver was found to be accumulative and directly proportional to the dose. Silver was still retained 2½ years after the last injection of the drug.

In similar studies involving the treatment of lupus erythematosus with gold sodium thiosulfate, Gaul and Staud (1933) established that a dose of 0.1 gm. of the salt was sufficient to produce detectable amounts of gold in the subsequent spectrographic analysis of skin samples. Furthermore, the total amount of gold in the skin was found to be proportional to the total dose of the drug. The deposits appeared to be uniformly distributed in the cutis and retained there permanently.

In another publication Gaul and Staud (1935b) reported that spectrographic analysis of sputum, feces, and urine showed approximately equal amounts of gold excreted through the lungs and the gastrointestinal tract and only a trace through the kidneys. On the basis of the data available, a discussion was presented concerning the physiopathologic disposition of the metal, the mode of excretion, and the manner in which the metal is distributed in the body as a reciprocal of the capillary system. A similar study has been made for silver by the same authors (1935c).

Further studies on patients receiving various amounts of gold sodium thiosulfate for lupus erythematosus were reported in 1944 by Beinhauer, Jacob, and Beebe. Spectrographic data were obtained, over a period of eight years, to determine the presence or the absence of gold in normal skin, in active lesions, in healed lesions, and in blood and urine with relation to the time element. A summary of their results follows: (1) Gold was detected in every specimen from a healed scar (10 examined); (2) in 13 of 20 normal skin specimens from persons under treatment, gold was detected; (3)gold was not detected in active lesions (six examined); (4) gold was detectable in the blood from 10 min. until three weeks after injection, and (5) gold was detectable in urine from one hour to one week after injection. In the discussion following their report, one of the authors mentioned that deposits of bismuth, mercury, and arsenic were looked for in patients being treated for lupus erythematosus and syphilis. Very little deposit of these metals was found in the skin, even though the salts were administered therapeutically.

APPLICATIONS IN THE FIELD OF PATHOLOGY

Some of the earliest applications of emission spectroscopy in medicine were those in cases of metal poisoning. Since the information desired under such circumstances is frequently semiquantitative (order of magnitude), the method can be applied without the time-consuming procedures required in quantitative work.[5]

During a series of lead-poisoning cases in Baltimore in 1932, rapid diagnosis was particularly important for individuals without clinical manifestations. Shipley, Scott, and Blumberg applied spectrographic techniques to the problem. A 5-cc. blood sample was ashed with sulfuric acid, ignited in a muffle furnace, and then excited on graphite electrodes in the direct-current arc. Appreciable quantities of lead were found in nine cases of poisoning, but only traces or none were found in samples from normal individuals. Later (1934, 1935a, 1935b), Blumberg and Scott reported values for blood lead and the associated clinical condition (Table 4). Further observations by these authors were that during convalescence and in absence of additional exposure the clinical symptoms disappear before the blood level falls to a nonpathological level; and pathological blood-lead values persist six to 12 months after exposure, especially in children.

The same authors (1935c) applied their spectrographic method to determine the blood-lead location. In studies on 18 cases of clinical lead poisoning they noted that about 90% of the blood lead was found in the clot and concluded that, unlike

5. Cavanna and Angeleri. Cholak, 1935a, 1935b, 1937, 1944. Cholak, Hubbard, McNary, and Story. Esser. Foster, Langstroth, and McRae, 1935. Parva. Patterson. Peterson, Welford, and Harley. Piccardi. Rankin. Sawyer, Waggoner, and Erickson. Schonlebe. Schrader, Steadman, and Thompson. Strait. Urbrandt and Francone, 1940a, 1940b. Vigliani, 1938, 1940. Vigliani and Deaglio.

calcium, lead is carried in the cellular elements of the blood rather than in the serum. Analyses were made on the separated fractions from oxalated, citrated, and heparinized samples, as well as on serum and clot. Samples of control bloods indicated that the cell fraction contained at least half of the blood lead. Washing the cells with isotonic sodium chloride solution failed to remove appreciable quantities of lead, demonstrating a rather firm combination of the lead and red blood cells.

Rabinowitch, Dingwall, and Mackay centered their lead studies on the analysis of cerebrospinal fluids. They described in detail the procedure for collecting specimens with minimum chance of contamination from spinal puncture needles, glassware, reagents, and dust. The direct-current-arc method is sensitive to 10^{-5} mg. of lead. With the procedure using 6-cc. quantities of cerebrospinal fluid they could detect lead present in concentrations as low as 1 in 1,000 million parts of fluid.

Kehoe examined several methods of lead determination in relation to the diagnosis of lead poisoning and recommended without reservation the spectrographic method for its accuracy, sensitivity, speed of manipulation, and the apparent universality of its application to all types of material. He cautioned against some of the common means of contamination under prevailing hospital conditions, such as needle, syringe, and catheter contact, and activity of the patient during the period

TABLE 4.—*Correlation of Blood Lead with Pathological and Clinical Manifestations*

		Mg./100 Cc.
Nonpathological (clinically negative)	"Normal" lead	0.005-0.05
	Slightly abnormal lead exposure	0.05-0.1
Pathological borderline (clinically questionable)	Subclinical or mild clinical lead poisoning	0.1-0.2
Pathological (clinically positive)	Lead poisoning or convalescence	0.2-1.0
Critical range for appearance of symptoms		0.1-0.2

preceding and during the collection of the sample. Spectroscopically, he found the lead concentration in the blood of normal persons to vary from 0.01 to 0.12 mg. per 100 cc., with a mean value of 0.06 mg.

Armstrong and Brackett in 1939 published a discussion of a procedure involving the direct-current arc for the analysis of lead, manganese, and mercury in body fluids, urine, and blood. The method was developed for the purpose of detecting small quantities of toxic materials in cases of industrial exposure.

During a medical and engineering study of the storage-battery industry by the Public Health Service in 1937-1938 to ascertain the effect of working conditions on health, spectrographic methods [6] were used to determine urinary lead concentrations. Results obtained spectroscopically were in agreement with those obtained by chemical methods. The urinary mercury values reported in two other government surveys [7] were also obtained by spectrographic methods.

Tracy and McPheat analyzed blood for lead by a spectrographic method using platinum as the internal standard. They found that lead is transmitted to the fetus in amounts approximating those in the maternal circulation and that lead is present in human milk and is very slowly excreted in the urine of infants.

In the interest of industrial hygiene, Cholak and Hubbard (1946) developed a spectrographic method for determining antimony in biological material. The

6. Armstrong, 1941a, pp. 83-86.
7. Armstrong, 1937; 1941b, pp. 106-110.

procedure required wet-oxidation, isolation of antimony as sulfide, excitation by arc and photometry of unknown and internal standard lines by conventional methods. The same authors (1948a, 1948b) also described a method for determining beryllium in biological materials and in air.

Barnes, Piros, Bryson, and Wiener, in 1949, described direct-current-arc procedures for determining beryllium in urine in quantities as low as 0.04 γ per liter. They have also applied the method to tissue and air samples.

Gerlach and Gerlach [8] were especially interested in the pathological application of spectrographic methods and reported results for a number of cases. In their method known as histospectrography, dried histological sections were placed on a glass plate and excited with a high-frequency spark as previously described.[1] These workers could demonstrate silicon in the lungs in cases of pneumoconiosis. They also found metallization of tissues accompanying electrical injuries. The histospectrographical technique was used in forensic medicine by Gerlach and Gerlach to distinguish between the points of entry and exit of bullets by analysis of the injured tissue. This method is ingenious, but unfortunately it is not suitable for quantitative analysis.

Other workers have studied the presence of silicon in silicotic lungs using semiquantitative methods. Scott and Williams (1934, 1935) used spark analysis, and Ramage (1929, 1936) and Sheldon and Ramage (1931) burned dried organs in an oxycoal-gas flame. Both methods have shown high concentrations of silicon and iron in cases of silicosis (Richter).

Ramage (1929) mentioned that his method of flame analysis had been early applied to the examination of normal and anemic blood. He reported only that distinct differences were noted in the iron, calcium, magnesium, and potassium contents.

Policard, Morel, and Ravault [9] have applied histospectrographic methods to analysis regarding calcium and magnesium in normal and atheromatous portions of human aortas. The analysis was based on the intensities of persistent lines as compared with standard solutions. The specimens were from four patients between 50 and 70 years of age with arteriosclerotic lesions of the aorta. Using microdissection techniques, they separated intima, media, and adventitia and analyzed each of the two inner coats for calcium and magnesium. They concluded that in the normal portions of the aorta, both the intima and the media contained more calcium than magnesium, the calcium and magnesium levels being proportionally higher in the media. In the atheromatous areas there was an increase of calcium but no increase of magnesium, while in the media underlying the atheromas there was a decrease of the calcium and magnesium, but proportionately the decrease of magnesium was apparently more marked than that of calcium. When the hard calcified arteriosclerotic plaques were analyzed, an increase was found in the calcium but not in the magnesium.

Using similar methods, Policard, Bonnet, and Bonamour analyzed the eye in a case of Wilson's disease and found traces of copper in the region of the Kayser-Fleischer corneal ring but none in the anterior portion of the cornea, the ciliary body, and the lens. They also reported absence of iron, zinc, and silver. Cowdry,

8. Gerlach, 1933, 1933-1934, 1939. Gerlach and Gerlach, 1931, 1934a, 1934b, 1938. Gerlach and Ruthardt. Gerlach, Ruthardt, and Prusener.

9. Morel, Policard, and Ravault. Policard, Morel, and Ravault.

Heimburger, and Williams used a spectrographic method for analyzing leprous lesions of the skin and showed that there was an increase in the phosphorus/calcium ratio as compared with normal controls. The sodium/calcium and magnesium/calcium ratios were not altered. Similarly, MacCardle, Engman, and Engman found that in cases of chronic neurodermatitis both the active lesions and the unaffected skin contained a decreased amount of magnesium.

Gaul and Staud (1934b) applied the same techniques as used in their therapeutic studies to cases of psoriasis. In preliminary analysis they examined 300 normal skin specimens to establish the incidence of nickel. They found nickel in 6% of adult samples and in none from infants. In tissue from the central portions of psoriatic lesions from 26 patients, nickel was found in every case. The amount was reported as between 0.00001 and 0.00009 gm. per 0.025 gm. of specimen.

Schwartz and Fraser reported a case with many of the histologic features of psoriasis. The lesions contained excessive quantities of nickel and lead as determined by Gaul and Staud. A demineralizing diet and medication resulted in rapid healing of the ulcers. A second analysis showed the metallic elements in normal quantities except for nickel, which was unaltered by the treatment.

In a qualitative study of the metals in specimens diagnosed as "cancer," myoma, sarcoma, lymphoma, papilloma, gallstones, and kidney stones, Okajima (1931a, 1931b, 1931c) sparked ashed samples on aluminum, carbon, and copper electrodes. Both normal and diseased tissues were found to contain copper, manganese, iron, zinc, sodium, potassium, calcium, and magnesium. Silver was found in seven of 13 specimens of myoma, in eight of 18 specimens of "cancer" and in one or two specimens of papilloma. Aluminum was not detected in any of the specimens.

Dingwall and Beans, in a systematic study of the inorganic constituents of human tumors, examined 59 specimens of carcinoma and one of fibroma for the presence of chromium and molybdenum. The samples were oxidized with nitric acid and the solutions dried on carbon electrodes before excitation in the direct-current arc. Five specimens of carcinomas and one of fibroma contained molybdenum; six specimens of carcinoma contained chromium. With one exception, the metals did not occur together in tumors, but the authors were unable to explain the role of either when present.

Addink used the direct-current arc and the method of addition on low-temperature dry-ashed specimens to determine major and minor mineral constituents of the blood and other tissues in normal individuals and in patients with neoplasms. The author was particularly interested in searching for differences in composition of material originating from healthy people and from tumor patients and offered the following results as a contribution to the solution of the cancer problem. Semiquantitative analyses showed the greatest differences for zinc in the composition of blood and tissues from normal and tumor patients, with major element concentrations apparently unchanged. Table 5 demonstrates the significant differences of zinc in blood and liver.

In a procedure designed to handle original body fluids by spraying the fluid onto the surface of the electrodes of a carbon spark, Cassen found blood-plasma magnesium values of 3 to 4 mg. per 100 cc. and whole-blood magnesium values of 6 to 7 mg. per 100 cc. The average magnesium value for cerebrospinal fluid was determined as 3 mg. per 100 cc. One case examined by the author demonstrated an increase in magnesium content of the spinal fluid. The patient was an

infant with atrophy of the cortex of the brain, enlargement of cervical lymph nodes, and high serum phosphatase. No definite significance was assigned to the author's finding except that it appeared to be the first on record of an abnormal magnesium content of the cerebrospinal fluid.

Since the flame photometer became commercially available, it has found increasing use in the field of clinical pathology and experimental medicine. This instrument is of great value in the study of electrolyte balance, especially in the determination of sodium and potassium, in view of the speed, simplicity of operation, and the relatively high precision inherent in it as an analytical tool. Publications on methods, procedures, and applications are appearing in increasing numbers in the literature.[10] Reference has already been made to the work of Cholak and Hubbard (1944b), Hald, Overman and Davis, and Robinson, Newman, and Schoeb. In a recent publication, C. L. Fox presented a thorough discussion of the methods of flame photometry, including a comparison of instruments, sample preparation, sensitivity, and sources of error in determining sodium and potassium in plasma, urine, and tissues. He cited the importance of the method in research, diagnosis, and treatment.

TABLE 5.—*Zinc in Blood and Liver*

	Zinc Content Range	Standard Deviation
Blood		
No disease	0.0002-0.0010%, mean 0.0007%	0.0001%
Nontumorous disease	0.0004-0.02[a]%, mean 0.0012%	0.0002%
Cancer	0.0004-0.005[a]%, mean 0.0016%	0.0006%
Liver		
Nontumorous disease	0.008[a]-0.008%, mean 0.005[a]%	0.0005%
Neoplasms	0.004 -0.013%, mean 0.007[a]%	0.0012%

Respecting superior 3, 0, and 5, the superior value was determined, but the significance is questionable.

Marinis, Muirhead, Jones, and Hill used the flame photometer to determine the sodium and potassium levels in the serums of 107 normal adults and cited seven cases in which the sodium and potassium determinations were used to detect deficient or dangerously high potassium concentrations and to follow the return to normal with medication. The serum sodium values for normal individuals were found to range from 135.5 to 153.2 mEq. per liter, mean 144.0; potassium values were 3.1 to 6.2 mEq. per liter, mean 4.52.

From the volume of reports in the recent literature it is apparent that as more laboratories have gained experience in the use of the flame photometer, the method has proved to be a valuable one for the routine laboratory as well as for clinical investigation.

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EXPERIMENTAL FOOD-ASPIRATION PNEUMONIA

THOMAS J. MORAN, M.D.
PITTSBURGH

FOOD-ASPIRATION pneumonia has not been widely recognized as a distinct clinical and pathological entity. This lack of recognition is due at least partly to the difficulty of detecting and identifying aspirated material in the pneumonic areas of involved lungs. Apfelbach and Christianson [1] in 1931 and Irons and Apfelbach [2] in 1940 stressed the importance of postoperative aspiration pneumonia. They based their belief that such pneumonias were caused by aspiration chiefly on the bacteriologic similarities of cultures of the stomach contents and of material from the trachea and bronchi and the pneumonic areas of the lungs. These authors stated that particles of undigested food might be found on microscopic examination of the lungs in such cases if enough sections were made.

In 1942 Fetterman and Moran [3] reported 27 instances of food-aspiration pneumonia encountered in a series of 469 consecutive autopsies in an institution where the average age at autopsy was 60 years and where both general hospital and mental hospital patients were included in the series. Our recognition of this condition was based on the finding of definite food particles in the areas of pneumonic reaction, and we noted that the reaction varied from an acute inflammatory process to a chronic granulomatous lesion with foreign-body giant-cell formation and fibrosis.

Because of this frequent finding of food particles in association with the pneumonias of elderly people I decided to produce food-aspiration pneumonia in animals in order to determine whether the pathological reactions were similar to those in man and to determine the approximate time intervals required for the various stages of the process.

METHODS

A variety of common cooked foods, including peas, corn, potatoes, bread, and "wieners," were injected intratracheally in 1-cc. doses into 60 healthy adult guinea pigs. The foods were not mixed, only one type of food being injected into each animal. The food was well ground, suspended in isotonic sodium chloride solution and filtered through a single layer of gauze before injection. Sterile technic and ether anesthesia were used with the first group of animals, but

From the Department of Pathology and the John C. Oliver Memorial Research Foundation, St. Margaret Memorial Hospital, and the Department of Pathology, University of Pittsburgh School of Medicine.

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later injections were made without anesthesia or strictly sterile precautions. One animal died in 24 hours. Others were killed by chloroform inhalation at periods ranging from a few minutes to two months. The animals were divided into 11 groups of five each, a group being put to death at intervals of 2 to 3 minutes, 1 hour, 2 hours, 6 hours, 12 hours, 24 hours, 48 hours, 1 week, 2 weeks, 1 month and 2 months. A few extra animals were given intratracheal doses where there was some doubt as to the success of the initial injection. At least three blocks were selected from each lung and fixed in 4% formaldehyde solution. Paraffin sections were stained by our routine hematoxylin-and-eosin method.

RESULTS

Changes essentially the same as those observed in human lungs, including both acute pneumonia and chronic granulomatous reactions, were found in these animals. All of the foods used produced the same changes in the lungs. Gross changes were nonspecific and varied from edema and congestion to nodular bronchopneumonia. In late stages small granulomatous or fibrotic nodules were noted in most lungs.

In animals killed in one hour or less, many food particles were found in the bronchi and the alveoli on microscopic examination, but definite pneumonia was not present. Edema and congestion were often striking, and a few segmented neutrophilic granulocytes were found around occasional food particles. In 2 to 12 hours acute pneumonia developed, and most of the food particles were completely and tightly surrounded by a thin rim of segmented neutrophils, one to three cells deep. Occasional food particles were partially degenerated, and in many of them the segmented neutrophils had penetrated the cell wall and were found within the particles, at times in considerable numbers. The rim of segmented neutrophils was in turn surrounded in 6 to 12 hours by a layer of other inflammatory cells, chiefly monocytes and lymphocytes. Giant cell formation was noted in a few cases as early as 12 hours after injection of the food, and in 24 hours well formed giant cells were found in numerous instances. In many guinea pigs an extensive giant cell reaction was noted around food particles in 24 to 48 hours.

Numerous large granulomatous areas were noted at the end of a week. In many of these areas food particles were still intact, while in others they were partially degenerated or absent. Lungs of the guinea pigs killed at one to two months showed extensive patchy granulomatous areas many of which contained no recognizable food particles. Definite areas of fibrosis were noted at the end of two months.

A general pneumonic reaction occurred in addition to the local reaction immediately around the food. It developed in two to six hours and was made up of segmented neutrophils, edema fluid, red blood cells, lymphocytes, and monocytes. In some instances the pneumonia was limited to areas around the food particles, but in other instances it was quite widespread, extending considerably beyond the areas in which the food was recognizable. Monocytes and lymphocytes often predominated in the cellular reaction in two days to one week, and the pneumonia frequently became interstitial as well as alveolar during this time. Some of the pneumonic areas apparently disappeared, while others formed chronic granulomas which in some sections occupied as much as one-half of the lung tissue. Alveolar fibrosis occurred in many of the old pneumonic areas in one to two months.

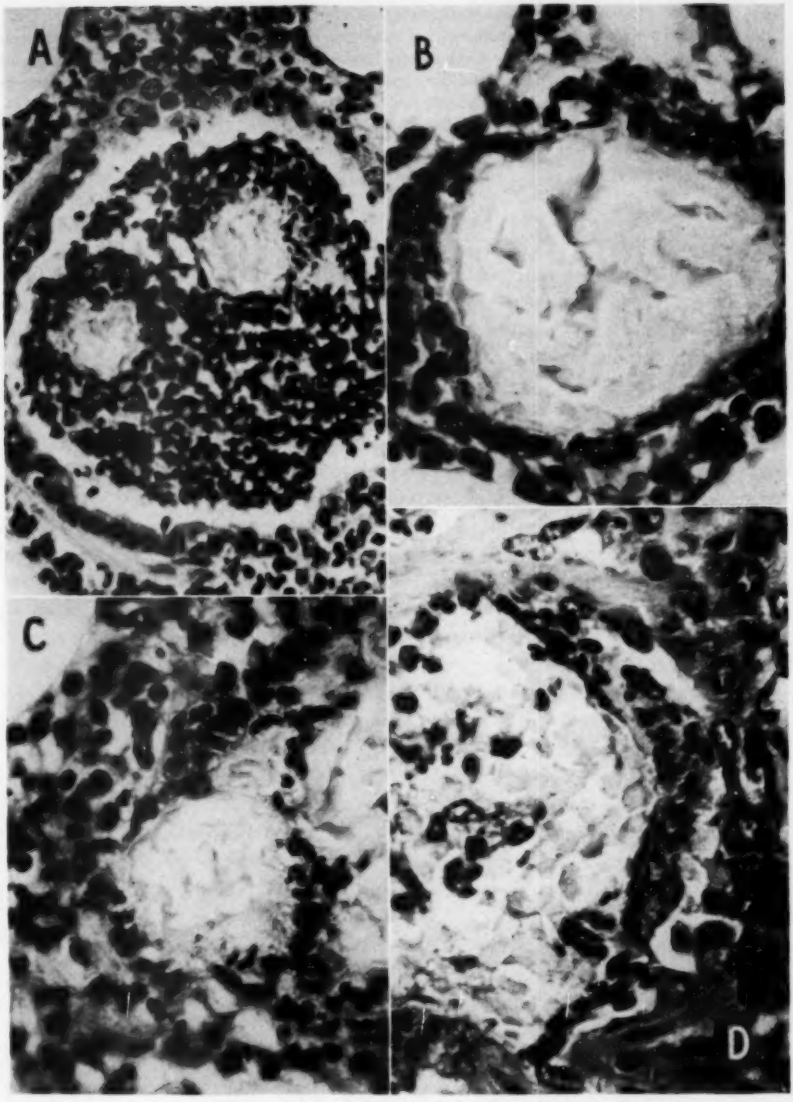

Fig. 1.—*A*, two starch cells surrounded by segmented neutrophils in a bronchiole. There is early acute inflammation around the bronchiole.

B, narrow rim of segmented neutrophils around a starch cell.

C, segmented neutrophils extending through the cell wall. A rim of segmented neutrophils immediately around the food is surrounded by a layer of monocytes and lymphocytes.

D, numerous segmented neutrophils inside degenerated food particle. A zone of monocytes and lymphocytes is seen on the right surrounding the rim of segmented neutrophils along the outside of the food particle.

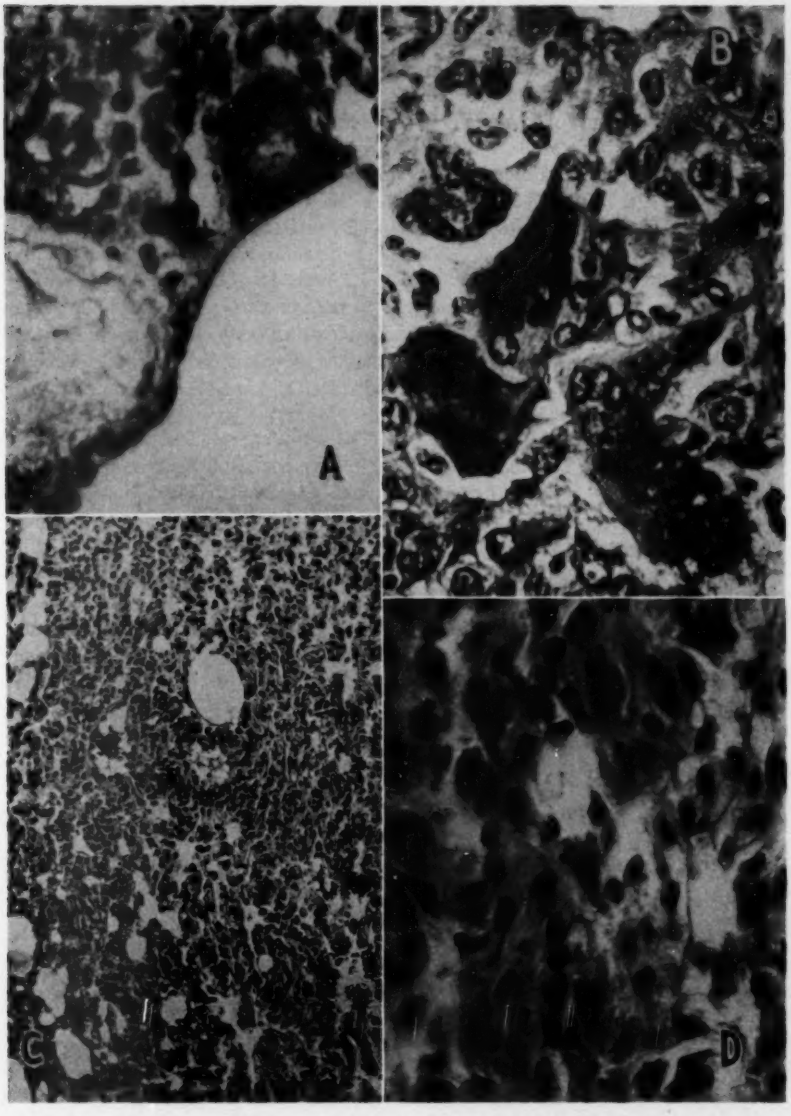

Fig. 2.—*A*, early granulomatous reaction around food with formation of a foreign-body giant cell.

B, chronic granuloma showing large foreign-body giant cells but no food. Easily recognizable food particles were found in sections deeper in the block.

C, low-power view of a fibrotic area.

D, high-power view of a fibrotic area. No food is seen in this section, but other sections show typical food particles.

COMMENT

Microscopic recognition of the food in the lung may be as difficult in the guinea pig as it is in human cases of food-aspiration pneumonia. In general, the starchy foods, such as peas and beans, are easy to identify as food, because of the characteristic starch granules in the cells. But material such as bread, carrots, and celery may be difficult or impossible to identify as food because of the lack of characteristic structure. At times bits of epidermis from plant foods may be seen in the sections, and meat can be identified by the finding of skeletal muscle. Considerable information regarding the microscopic appearance of various foods was gained by fixing many of the common foods in 4% formaldehyde solution, sectioning, and staining the sections by routine hematoxylin-and-eosin methods. The similarity in appearance between certain known food particles in the guinea pig lungs and material in human lungs which could not be definitely identified as food suggests that food particles which are not recognized as such may be more common in routine sections of human pneumonias than is generally realized.

SUMMARY

The experimental pneumonia produced in a series of 60 healthy adult guinea pigs by intratracheal injections of 1-cc. amounts of various cooked foods has been described. This reaction varied from an acute pneumonic process to a chronic granulomatous lesion with giant cell formation and fibrosis.

The reactions to food produced in the guinea pig were almost identical with those observed in food-aspiration pneumonias in man. An important similarity was the finding of fairly large pneumonic areas in which definitely recognizable food particles were absent. This finding stresses the necessity of studying multiple sections from various areas in instances in which food-aspiration pneumonia may be suspected. This is particularly true of chronic granulomas of undetermined cause, where one section may reveal only a nonspecific reaction with foreign-body giant cells and a section deeper in the block may show definite, easily recognizable food particles. Food-aspiration pneumonia should be considered as a possible etiologic factor in any chronic granulomatous or fibrotic lung lesion of unknown cause.

It is hoped that this description of the pathologic processes found in food-aspiration pneumonias in guinea pigs will focus attention on the need to recognize this disease in human subjects.

CEREBRAL DEMYELINATION AND FOCAL VISCERAL LESIONS IN A CASE OF ACUTE HEMORRHAGIC PANCREATITIS

With a Consideration of the Possible Role of Circulating Enzymes in the Causation of the Lesions

F. STEPHEN VOGEL, M.D.
NEW YORK

WIDESPREAD cerebral demyelination and focal lesions of the heart, the adrenals, and an ovary were found in the case of acute hemorrhagic pancreatitis now to be reported. The findings, considered in relation to recent experimental observations,[1] suggest that enzymes liberated from the damaged pancreatic tissue may have initiated the cerebral and visceral lesions.

CLINICAL OBSERVATIONS

A white woman, 44, entered the hospital because of headaches that had accompanied severe arterial hypertension for more than three years. For six months she had experienced increasing exertional dyspnea, occasional nocturnal dyspnea, and minimal edema of the ankles.

The positive physical findings were confined to the cardiovascular system: The blood pressure was 260/160; the vessels of the ocular fundi showed marked arteriovenous nicking; the heart size by x-ray film was at the upper limit of normal, and the electrocardiogram revealed left axis deviation with evidence of coronary-artery disease. The urine had a specific gravity of 1.011 and contained a trace of protein. The blood urea nitrogen was 10 mg. per 100 cc.

A right thoracolumbar sympathectomy was performed, and three weeks later a similar procedure was carried out on the left. After the second operation the patient did not fully regain consciousness, and examination on the next day showed areflexia with complete flaccidity of the muscles of the face and the extremities. A tender epigastric mass was palpable for the first time just to the left of the midline. The blood pressure, which had been 280/150 prior to the second operation, was 110/70. There was oliguria, and the blood urea nitrogen rose to a terminal level of 61 mg. per 100 cc. Death occurred 72 hr. after the operation.

NECROPSY

Postmortem examination, performed four hours after death, showed the tumefaction in the epigastrium to be a large hemorrhagic mass associated with the distal portion of the body and the tail of the pancreas, and the fat of the mesentery and the omentum showed much hemorrhage and necrosis. On histologic examination, the parenchyma of the pancreas, including Langerhans' islets and the interstitial tissues, was extensively necrotic, and there were widespread fat necrosis and much hemorrhage in the peripancreatic tissues. The involved areas were densely infiltrated by granulocytes and phagocytic histiocytes (Fig. 1*A*).

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From the Department of Pathology of the New York Hospital, Cornell Medical Center, New York.

1. (*a*) Vogel, F. S.: J. Exper. Med. **93**:297, 1951. (*b*) Robb-Smith, A. H. T.: Lancet **2**:362, 1945. (*c*) Robertson, T.; Kellner, A., and Thal, A., in Proceedings of the American Association of Pathologists and Bacteriologists, Am. J. Path. **27**:753, 1951.

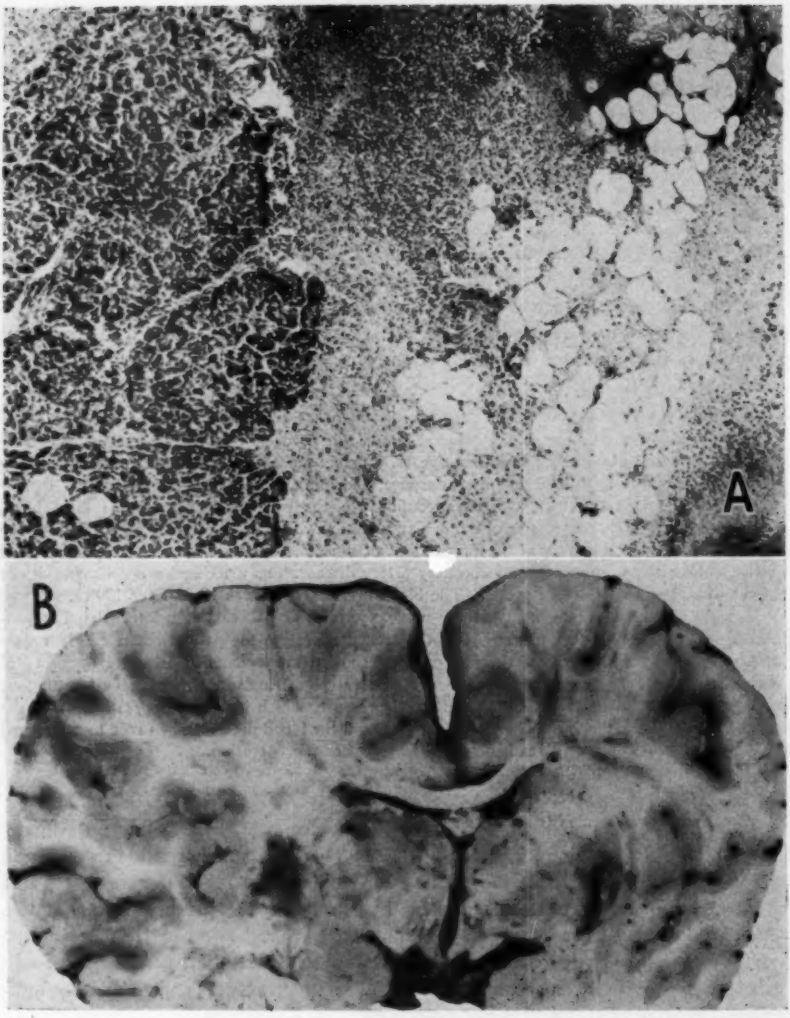


Fig. 1.—*A*, acute hemorrhagic pancreatitis. Characteristic necrosis of fat and parenchymal tissue is widespread and accompanied by extensive hemorrhage and marked leukocytic infiltration. × 100.

B, coronal section of the brain showing that the left cerebral hemisphere is notably larger than the right and that the ventricular system is displaced laterally. There are mottling and discoloration, most conspicuous in the gray matter of the left middle and inferior frontal gyri. × 0.8.

The brain weighed 1,350 gm. The left cerebral hemisphere was notably larger than the right, and the ventricular system was displaced laterally toward the right. The gyri of the left frontal and parietal lobes were flattened, and over them the pia-arachnoid was opaque and slightly thickened. There was fulness of the cerebral and pial veins. The arteries at the base of the brain were patent and showed only a moderate degree of atherosclerosis. After one week's formaldehyde solution-bromide fixation [2] the gray matter, and to a lesser extent the white matter, appeared mottled with small patchy areas, 0.5 to 3 cm. across, where the cerebral tissues were a homogenous dusky yellowish-gray color. A few lesions contained punctate hemorrhages, but nowhere was the cerebral tissue friable or necrotic. The changes were most conspicuous in the gray matter of the left frontal and parietal lobes but were to be seen also in the right cerebral hemisphere and basal ganglions but not in the brain stem, the medulla or the cerebellum (Fig. 1*B*). The spinal cord was not examined.

Histologic sections from many areas in both cerebral hemispheres showed diffuse, patchy loss of myelin when stained specifically for this substance by the Weil method; these changes were not conspicuous in routine preparations stained with hematoxylin and eosin. The gray matter was more extensively involved than the white matter, but often the myelin was absent or pale-staining in small and large, irregular, confluent areas that involved contiguous portions of gray and white matter (Fig. 2). Often the loss of myelin was perivascular in distribution (Fig. 2*C*). The nerve cells showed varying degrees of chromatolysis and hyperchromatism, but as seen in the Nissl and Cajal preparations they and their axon processes were generally well-preserved. Silver carbonate preparations demonstrated no notable increase in the number of astrocytes, and the microglia were not significantly altered. Noteworthy was the regular absence of inflammatory cells. A few small perivascular hemorrhages were present, but the blood vessels were patent throughout and showed only slight fibrous thickening of their walls. Sudan III preparations failed to demonstrate intravascular fat emboli in the brain, and similarly negative results were obtained when the lungs were examined in like manner.

The heart weighed 470 gm. and showed moderate hypertrophy of the left ventricular wall. The coronary arteries were patent, and there were only small atheromatous plaques within their walls. The myocardium contained scattered minute grayish-red streaks; these on histologic section were formed of focal groups of muscle cells in which there were a striking loss of sarcoplasm and an absence or pyknosis of nuclei. In contrast, the reticulum and collagenous interstitial tissues remained intact and appeared as delicate honeycomb structures that were slightly infiltrated by lymphocytes and small mononuclear phagocytes (Fig. 3*A*).

The adrenals were not grossly altered. Nevertheless, as was evident in histologic sections, the cortex of each contained several diffusely scattered areas where the nuclei of the parenchymal cells were shrunken and hyperchromatic and the cytoplasm stained poorly or not at all. Peripherally about these lesions there were sparse lymphocytic and granulocytic infiltrations (Fig. 3*B*). Although the changes

2. Formaldehyde solution U. S. P. (420 cc.), ammonium bromide (60 gm.) and water to make 3,000 cc.

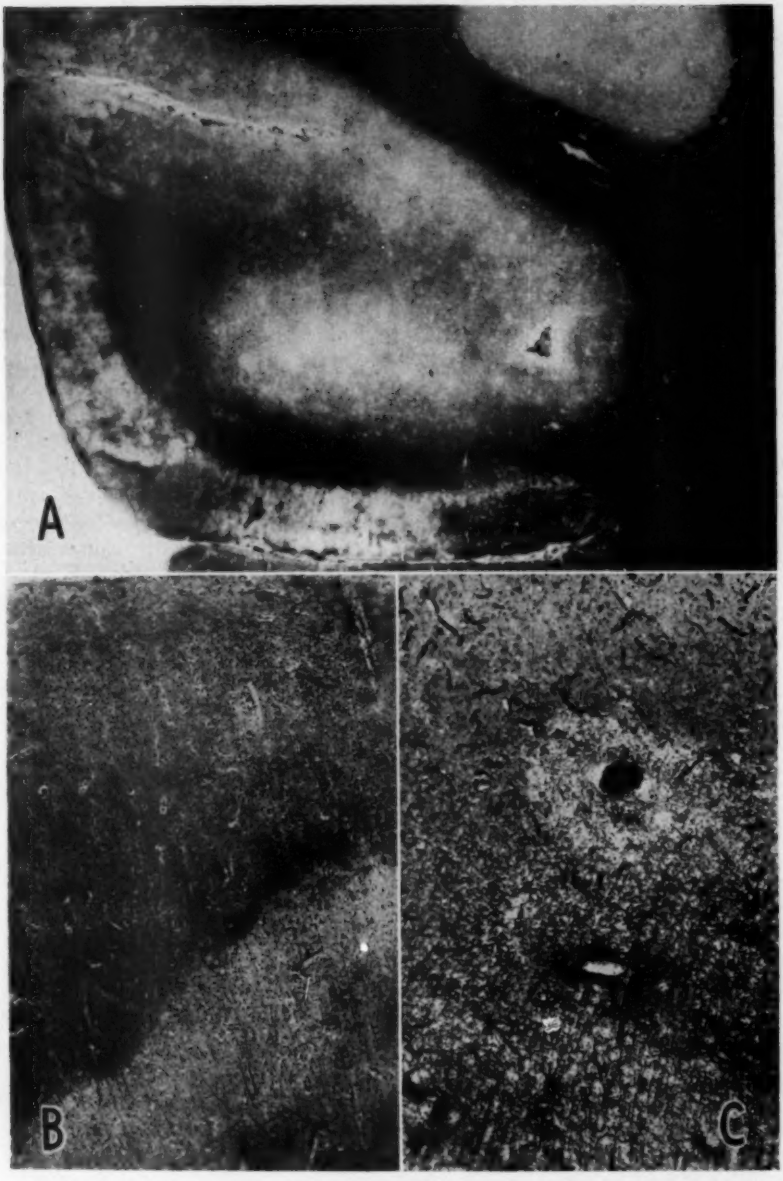


Fig. 2.—*A*, Weil preparation of the left middle frontal gyrus, showing extensive, diffuse, patchy demyelination of the gray and the white matter. × 5.

B, a sharply delineated area of demyelination in the cerebral gray matter. The absence of inflammation and the preservation of the nerve cells are noteworthy. Weil preparation; × 70.

C, loss of myelin about a large vessel in the gray matter. The white matter beneath shows demyelination. Weil preparation; × 100.

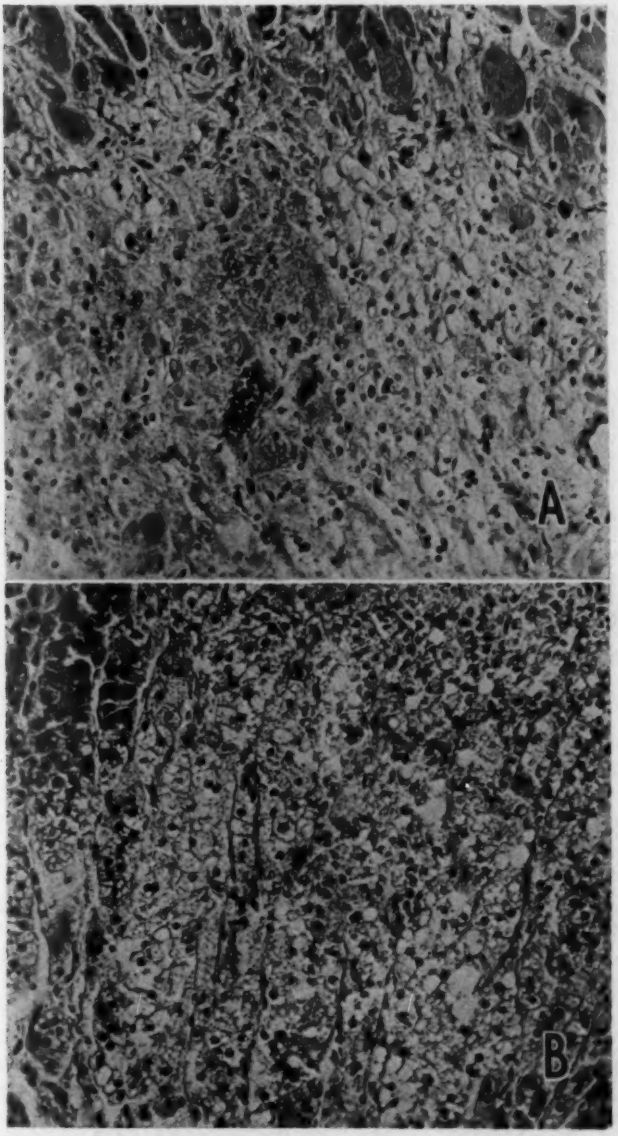


Fig. 3.—*A*, focal lesion in the myocardium of the left ventricle chararacterized by a dissolution of sarcoplasm and pyknosis or absence of the muscle nuclei. The interstitial collagen is preserved. Hematoxylin and eosin stain; × 200.

B, focal lesion in the adrenal cortex in which the cytoplasm of the parenchymal cells is pale-staining and granular and the nuclei are pyknotic. Granulocytic infiltration is slight. Hematoxylin and eosin stain; × 200.

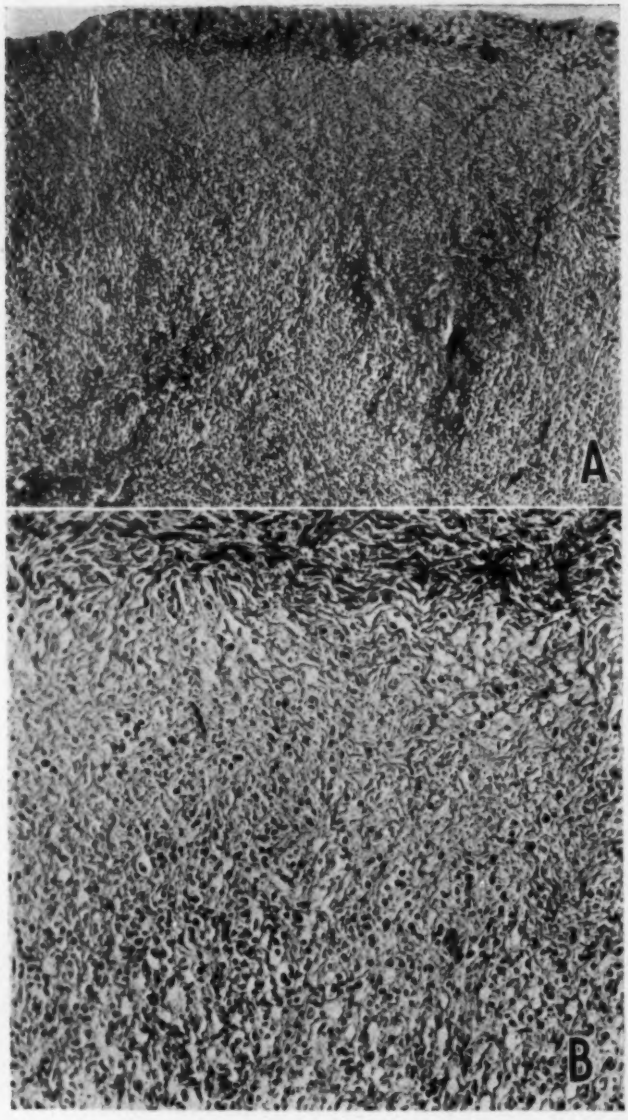


Fig. 4.—*A*, wedge-shaped lesion in the ovarian cortex that extends peripherally but does not involve the tunica albuginea. Hematoxylin and eosin stain; × 75.

B, higher magnification showing the ovarian lesion to be characterized by pyknosis of the stromal cell nuclei and pale-staining or dissolution of their cytoplasm. Hematoxylin and eosin stain; × 200.

were not unlike those attributed to shock,[2a] other viscera showed no stigmata of this condition.

Both ovaries were somewhat atrophic. Only the right was studied histologically. Within its cortex there were several discrete pyramidal lesions, 0.5 to 1 cm. across, in which the cytoplasm of the stromal cells was swollen, amorphous, and deeply eosinophilic and the nuclei were absent or pyknotic. The lesions contained only slight lymphocytic infiltrations (Fig. 4).

The remainder of the viscera were free from significant gross and microscopic lesions.

COMMENT

Recent reviews of the literature on acute hemorrhagic pancreatitis [3] and on cerebral demyelination [4] make no mention of a concurrence of these two disease processes. It is impossible, of course, to say whether the present case is unique in this association or whether in previously examined cases of acute pancreatitis cerebral demyelination may have been overlooked. In this regard certain facts deserve mention: Hemorrhagic pancreatitis usually runs an acute course, so that cerebral demyelination would have little time to declare itself clinically; indeed, the clinical aspects of the present case were unusual because of the presence of areflexia. Furthermore, the cerebral lesions in this case were not evident on gross examination of the fresh brain but became conspicuous only after prolonged formaldehyde solution-bromide fixation, and although widespread and readily demonstrated by means of special stains, they were not apparent in sections stained with hematoxylin and eosin in the routine fashion.

As already mentioned, the cerebral lesions in the present case were characterized by numerous, widespread focal areas of demyelination with little neuronal destruction and no inflammation, and in these respects were identical with the cerebral lesions recently induced experimentally in animals by means of a pancreatic lipase preparation.[1a] The perivascular distribution of the cerebral lesions and their greatest frequency in the more highly vascularized gray matter both suggest that some myelolytic substance was carried by the blood and diffused outward from the vascular bed to destroy the nearby myelin sheaths. This sequence of events necessarily occurred under the experimental conditions where lipase was injected directly into the carotid artery,[1a] and a similar mechanism has been postulated as the mode of action of the "endogenous toxins" reputedly responsible for the rare occurrence of cerebral demyelination coincident with hepatic disease, uremia, porphyria, and eclampsia.[5]

The focal lesions in the myocardium, the adrenals, and the ovary were characterized by dissolution of protoplasmic structures accompanied with slight or moderate inflammation. The possibility that these lesions might have been initiated by enzymes is strengthened by two experimental studies. Robb-Smith [1b] has shown by means of in vtiro experiments that trypsin can dissolve myoplasm while leaving reticulum and collagen more or less intact, and it seems noteworthy that the changes

2a. Moon, V. H.: Am. J. Path. **24**:235, 1948.

3. Taylor, R. A. R.: Ann. Roy. Coll. Surgeons England **5**:213, 1949.

4. Multiple Sclerosis and the Demyelinating Diseases, A. Res. Nerv. & Ment. Dis., Proc., Vol. 28, Baltimore, Williams & Wilkins Company, 1950.

5. Baker, A. B., in Multiple Sclerosis and the Demyelinating Diseases,[4] Chap. 6, pp. 59-74.

he induced in striated muscle were quite similar to those in the myocardium in the present case. Furthermore, a myocarditis characterized initially by focal lysis of muscle tissue has recently been induced by Robertson, Kellner, and Thal [1c] in living rabbits, rats, and mice by the intravascular injection of a proteolytic enzyme, papain.

SUMMARY

Widespread acute demyelination of the gray and white matter of the brain and focal lesions of the myocardium, the adrenals, and an ovary associated with acute hemorrhagic pancreatitis are here described. The possibility is considered that the demyelination and the visceral lesions may have been caused by excessive amounts of enzymes liberated from the damaged pancreas and circulating in the blood.

CHONDROMUCINASE FROM BOVINE TESTIS AND THE CHONDROMUCIN OF THE UMBILICAL CORD

R. D. LILLIE, M.D.
E. W. EMMART, Ph.D.
AND
A. M. LASKEY, B.S.
BETHESDA, MD.

THE OBSERVATION that the metachromatically staining connective tissue mucin of the umbilical cord and other structures can be digested by preparations containing the enzyme hyaluronidase has given rise to a widespread impression that this mucin is in fact hyaluronic acid. Meyer [1] and others have pointed out that smears and spots of isolated hyaluronic acid stain metachromatically with toluidine blue, though Meyer stated that solutions less concentrated than 1% failed to stain in his hands, and said that hyaluronic acid is probably never present in sufficient concentration to contribute to metachromatic staining and that the tissue metachromasia is probably due entirely to the chondroitin sulfate mucins. Sylven [2] even writes in 1951 that he considers hyaluronic acid nonmetachromatic. Hale [3] stated that "hyaluronic acid and similar polysaccharides, which probably contain no sulphate and do not stain metachromatically," are not satisfactorily demonstrated by toluidine blue. He based this statement on a comparison of preparations digested, between Carnoy fixation and paraffin embedding, by a streptococcus hyaluronidase and undigested similar preparations, observing a decrease in the ferric ion uptake of the umbilical cord matrix in the digested preparations, which ion uptake he attributed to hyaluronic acid. Hale prescribed Carnoy fixation, stating that hyaluronic acid is not preserved by aqueous formaldehyde solutions, and Grishman [4] also insisted on the use of fixatives of high alcohol content "because of the high solubility of hyaluronic acid in water."

It is well known that the metachromatically staining mucin of the umbilical cord is well preserved by a variety of aqueous fixatives. Lillie [5a] reported the use

From the Laboratory of Pathology and Pharmacology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, United States Public Health Service, Federal Security Agency.

1. Meyer, K.: Biological Significance of Hyaluronic Acid and Hyaluronidase, Physiol. Rev. **27**:335, 1947.

2. Sylven, B.: Personal communication to one of the authors (R. D. L.).

3. Hale, C. W.: Histochemical Demonstration of Acid Polysaccharides in Animal Tissues, Nature, London **157**:802, 1946.

4. Grishman, E.: Histochemical Study of Mucopolysaccharides in Various Normal Tissues, Bull. Internat. A. M. Mus. **28**:104, 1948.

5. (*a*) Lillie, R. D.: Studies on the Histochemistry of Normal and Pathological Mucin in Man and in Laboratory Animals, Bull. Internat. A. M. Mus. **29**:1, 1949. (*b*) The formula for alcoholic lead or mercury-formalin® solution is as follows:

Lead nitrate or mercury bichloride	8 gm.
Alcohol (100%)	80 ml.
Formalin® (40% formaldehyde)	10 ml.
Distilled water	10 ml.

The formaldehyde solution may be omitted and replaced by an additional 10 ml. of distilled water.

of aqueous formaldehyde solutions, but has had better results with aqueous 6% mercury bichloride, aqueous 8% lead nitrate, and 80% alcohol 8% solutions of mercury bichloride and of lead nitrate, each alone and with a 10% addition of 40% formaldehyde solution.[5b] Bunting [6] recently has used aqueous basic lead acetate, as have others.

Meyer [1] stated, on the basis of work in his own laboratory and in that of Madinaveitia and Stacey, that testicular hyaluronidase would hydrolyze corneal (sulfate) mucopolysaccharide, a chondroitin sulfate mucin from calf's tendon, and chondroitin sulfate from hyaline cartilage, and suspected the presence of another enzyme, since bacterial and leech hyaluronidases do not attack cartilage. He stated that acetone precipitation destroyed most of the chondroitin sulfatase without impairing the hyaluronidase activity. Bunting [6] and Lillie [5] have each reported a variably strong action of hyaluronidase in histochemical tests on the metachromatically staining component of cartilage matrix. Duran-Reynals, Bunting, and van Wagenen [7] postulated the presence of two enzymes. This duality is further supported by the noncorrespondence between the concentrations necessary to effect complete digestion of metachromatically staining material from umbilical cord and the stated turbidity-reducing-unit titers of separate lots of enzyme from the same and different manufacturers. With TRU titers varying from 14 to 1,500 TRU per milligram, concentrations required for cord digestion have varied only from 25 to 2 mg. per milliliter.

Meyer and Rapport [8] and Mathews, Roseman, and Dorfman [9] have both reported quite constant ratios between turbidimetric titers of testicular hyaluronidase concentrates against chondroitin-sulfuric acid from cartilage and against hyaluronate. Meyer and Rapport reported liberation of reducing sugars from suspensions of dried powdered cartilage. Mathews, Roseman, and Dorfman observed somewhat different effects of various metal ions on serum inhibition of the two activities. Both groups concluded that the two activities were assignable to a single enzyme.

In 1949 Lillie [10] and Lillie, Greco, and Laskey [11] reported the presence in barley-malt diastase of an enzyme which removed the metachromatically staining material from cartilage matrix. Like the ribonuclease from the same source and that from bovine pancreas, this enzyme resisted boiling in acetic acid solution. We have found also that digestion of umbilical cord sections in a 0.1% malt-diastase solution weakened metachromatic staining in four hours and completely

6. Bunting, H.: Distribution of Acid Mucopolysaccharides in Mammalian Tissues as Revealed by Histochemical Methods, Ann. New York Acad. Sc. **52**:977, 1950.

7. Duran-Reynals, F.; Bunting, H., and van Wagenen, G.: Studies on the Sex Skin of Macaca Mulatta, Ann. New York Acad. Sc. **52**:1006, 1950.

8. Meyer, K., and Rapport, M. M.: Hydrolysis of Chondroitin Sulfate by Testicular Hyaluronidase, Arch. Biochem. **27**:287, 1950.

9. Mathews, M. B.; Roseman, S., and Dorfman, A.: Determination of Chondroitinase Activity of Bovine Testicular Preparations, J. Biol. Chem. **188**:327, 1951.

10. Lillie, R. D.: On the Destruction of Cytoplasmic Basophilia (Ribonucleic Acid) and of the Metachromatic Basophilia of Cartilage by the Glycogen Splitting Enzyme Malt Diastase: A Histochemical Study, Anat. Rec. **103**:611, 1949.

11. Lillie, R. D.; Greco, J., and Laskey, A.: Histochemical Studies with Malt Diastase: Partial Separation of the Fraction Destroying Cytoplasmic Basophilia (Ribonucleic Acid) and the Metachromasia of Cartilage, Anat. Rec. **103**:635, 1949.

destroyed it in 18 hours at 37 C., while a control incubation for 18 hours in the buffer solution used as a solvent had no effect on subsequent staining with thionin.

While these findings point to the presence in umbilical cord matrix and arterial walls of a chondroitin-sulfuric acid mucin, it might be objected that malt diastase may also contain a true hyaluronidase. This question can be answered satisfactorily only by the separation of the two enzymes whose existence has been indicated.

EXPERIMENTS

Since the ribonuclease from barley-malt diastase and that from bovine pancreas resembled each other closely in their acid thermostability (Brachet[12]; Lillie and associates[11]), it was thought that a similar resemblance might be present between the chondromucinase from barley-malt diastase and that from bovine testis.

Accordingly, a 28.6 mg./100 ml. (20 TRU/ml.) solution of bull-testis hyaluronidase in 0.1 N acetic acid was boiled for five minutes.[13] After cooling, the solution was adjusted to pH 6.4 (nitrazine being used as an indicator) with a Beckman pH meter, by adding 1 N sodium hydroxide. A control raw-enzyme solution was made in the same manner except that heating was omitted. Paraffin sections of four umbilical cords, fixed severally in 8% aqueous lead nitrate, 80% alcohol 8% solutions of lead nitrate and of mercury bichloride, and aqueous 2% calcium acetate containing 10 parts of 40% formaldehyde solution per 100, were used as material. Sections were deparaffinized and hydrated as usual, employing the iodine thiosulfate sequence on the mercury bichloride material, and digested for varying intervals in the raw enzyme, in the acid-boiled enzyme, in 0.1 M acetate buffer of pH 6.4, in raw enzyme solution buffered to pH 5.5, and in enzyme solutions made up in buffers of pH 5.5 and 6.4 and boiled directly without acidifying. After digestion, all sections were stained 30 minutes in 1:2,000 thionin in pH 5.0, 0.01 M acetate buffer. With the raw enzyme and that boiled in acetic acid and then neutralized, complete digestion of both matrix and arterial mucin occurred in about the same time for each umbilical cord. With the control digestions in buffer solution and in the enzyme solutions boiled in pH 5.5 and pH 6.4 buffers the metachromatic mucin was as completely preserved as in the unextracted control sections.

Three further, similar experiments were made with bull-testis hyaluronidase. Concentrations of 25, 5, and 1 mg./100 ml. were used in two of these experiments[14]; 20, 4, 0.8, and 0.16 mg./100 ml., in the third.[15] The incubation times at 37 C varied from 2 to 16 hours. Umbilical cords were fixed in a variety of fixatives. In general, the raw enzyme seemed slightly more active than the acid-boiled, though on one series of trials arterial mucin was removed somewhat more promptly by the heated enzyme. The controls, using 16-hour extraction with the buffer solution alone, resulted in preparations which were quite comparable in mucin preservation to the preparations stained directly in thionin without digestion or extraction.

In parallel with the last two of these experiments, hyaluronidase activity of the same two lots of enzyme was determined by the turbidity reduction technic described by Kass and Seastone[16] and modified by Meyer,[1] Dorfman and Ott,[17] Tolksdorf and associates,[18] and others, with slight further variations. This method gives results which are reproducible within

12. Brachet, J.: La détection histochimique des acides pentosenucléiques, Compt. rend. Soc. biol. **133**:88, 1940.

13. The hyaluronidase used was Lot 9 HD 54-VIII-C, presented by the Schering Corporation.

14. In these two experiments Lot 9 HD 55-285-VI, 165 TRU/mg. donated by the Schering Corporation was tested.

15. In this experiment Lot W 173-A1, 1,500 TRU/mg. of Wyeth, Inc., was tested.

16. Kass, E. H., and Seastone, C. V.: Role of Mucoid Polysaccharide (Hyaluronic Acid) in the Virulence of Group A Hemolytic Streptococci, J. Exper. Med. **79**:319, 1944.

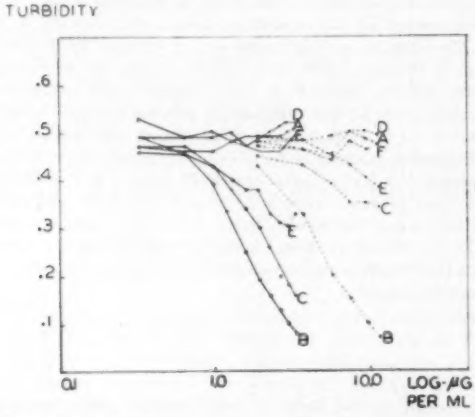
17. Dorfman, A., and Ott, M. L.: A Turbidimetric Method for the Assay of Hyaluronidase, J. Biol. Chem. **172**:367, 1948.

18. Tolksdorf, S.; McGready, M. H.; McCullough, D. R., and Schwenk, E.: The Turbidimetric Assay of Hyaluronidase, J. Lab. & Clin. Med. **34**:74, 1949.

a 10% experimental deviation (E. W. E.). Turbidity readings were taken with a Coleman spectrophotometer at 600/mμ.

Serial dilutions of enzyme solution in appropriate amounts to give a proper range to display gradation of activity of each preparation were deposited in tubes and warmed to 36 C. for five minutes, then diluted with phosphate-citric acid buffer of pH 5.5 (1.48 gm. $C_3H_4OH[CO_2H]_3 \cdot H_2O$+2.13 gm. Na_2HPO_4 per liter) to a volume of 1 ml. Then 1 ml. of 0.1% hyaluronic acid[19] solution in equal parts of 0.1 M sodium chloride and the above phosphate buffer was added to each tube, and the tubes were incubated at 36 C. in a water bath for 40 minutes. Then 10 ml. of 0.1% bovine-serum albumin (Armour fraction V) in pH 3.76 buffer (5 gm. glacial acetic acid +2 gm. sodium acetate $[NaCO_2CH_3 \cdot 3H_2O]$ per liter) was added to each tube, the tubes were shaken, and turbidity readings were taken on each at 30-second intervals.

The stock hyaluronidase solutions were prepared from the two aforementioned commercial lots.[20] Portion A was dissolved in 0.1 N acetic acid and heated to 70 C. in a water bath for 10 minutes and then adjusted to pH 5.5 with 4 N sodium hydroxide. Portion B was dissolved in 0.1 M acetate buffer of pH 5.5. Portion C was dissolved in 0.1 N acetic acid and adjusted to pH 5.5 with 4 N sodium hydroxide without heating. Portion D was dissolved in 0.1 M acetate buffer of pH 5.5 and heated to 70 C. for 10 minutes. Portion E was dissolved in 0.1 N



Curves of hyaluronidase reduction of hyaluronic acid (hyaluronidase of Wyeth, Inc.; hyaluronidase of Schering Corporation).

A. Enzyme was dissolved in M/10 acetic acid, heated, cooled, and adjusted to pH 5.5 with 4 N sodium hydroxide.

B. Enzyme was dissolved in M/10 acetic acid previously adjusted to pH 5.5.

C. Enzyme was dissolved at pH 3.75 and then adjusted to pH 5.5.

D. Enzyme was dissolved at pH 5.5 and then heated.

E. Enzyme was dissolved at pH 3.75.

F. Enzyme was dissolved at pH 3.75 and heated.

The enzyme concentration is logarithmically expressed in micrograms per milliliter (γ/ml.).

acetic acid (pH 3.75). Portion F was dissolved in 0.1 N acetic acid and heated to 70 C. for 10 minutes. Portions E and F were not neutralized.

The graphs *A*, *D*, and *F* in the accompanying chart indicate that the three heated portions were completely inert. Slight activity was shown by the unneutralized acid solutions (*E*), maximum activity by the solution (*B*) prepared in pH 5.5 buffer directly, without heating, and moderately impaired activity by the sample dissolved in acid and then neutralized (*C*). Similar

19. Lot JC-1-67 VI C, donated by the Schering Corporation.

20. Footnotes 14 and 15.

results were obtained with the two lots of enzyme, though the 1,500 TRU/mg. sample [15] required less concentration in micrograms per milliliter to hydrolyze the hyaluronate than did the other samples (165 TRU/mg.).[14]

Further digestion tests were made on a variety of other tissue sections, using the raw and the boiled hyaluronidase [15] solutions employed for the hyaluronic acid digestion and for the last series of umbilical cord digestions. Of the metachromatically staining materials tested, cartilage matrix and nucleus pulposus mucin were digested, while rat mast cell granules and epithelial mucins from rabbit pharyngeal gland, and rat and guinea-pig colons resisted the action of the enzyme.

Umbilical cords fixed with Carnoy's alcohol-chloroform-acetic acid fluid and with 2% calcium acetate, 10% aqueous formalin® were digested one, two, and four hours with raw and acid-boiled hyaluronidase [15] at 25 mg./100 ml. concentration, adjusted to pH 5.5. Two sets of controls were used, one unextracted, one extracted four hours in pH 5.5 acetate buffer (0.1 M). The preparations, after digestion, were stained in parallel with 0.05% thionin in 0.01 M acetate buffer of pH 4 for 30 minutes by the Hale [3] dialyzed iron technic and by the mannitol–ferric chloride, ferrocyanide procedure of Lillie and Mowry,[21] using a 30-minute immersion in mannitol–ferric chloride at pH 4.5.

As before, metachromatic staining of matrix and arterial mucin was greatly reduced or abolished even by the one-hour digestion period with both raw and acid-boiled enzyme. The Hale preparations exhibited an over-all deep-blue color in the controls, which was greatly reduced in all of the enzyme-digested preparations. Over-all coloration was deeper with the mannitol–ferric chloride procedure, but similar reduction of intensity appeared in the digested preparations. In both the Hale and the mannitol–ferric chloride preparation there was no appreciable difference in the over-all intensity of the Prussian-blue reaction between those digested with raw enzyme and those digested with enzyme which had been boiled in 0.1 M acetic acid and then adjusted to pH 5.5.

Histologic examination of iron-impregnated sections was less satisfactory than over-all gross inspection for determination of enzyme effect, because of the considerable iron impregnation of fine connective tissue fibrils by both technics, in agreement with the previous findings with the Lillie-Mowry technic. However, there is a very definite reduction of the interfibrillar blue staining, and the masses of coagulum in wide acellular spaces disappear.

COMMENT

The foregoing experiments would appear to indicate that testicular hyaluronidase contains at least two enzymes, a thermolabile one which acts specifically to depolymerize hyaluronic acid in vitro, and one which resists heating in acid solution and acts to abolish the capacity to stain metachromatically of certain connective tissue mucins and cartilage matrix. The former should be designated as true hyaluronidase, the latter probably as chondromucinase. This testicular chondromucinase, purified by thermal destruction of the associated hyaluronidase, appears to be about as active as the raw preparation containing both enzymes, in destroying metachromatic-staining capacity and in decreasing reactivity to ferric ion absorption as demonstrated in the Hale and the manitol-ferric chloride technics. From the lack of difference between the effect of the purified chondromucinase and that of its mixture with true hyaluronidase, one may argue that hyaluronic acid either does not react to the ferric ion absorption and metachromatic dye staining technics or is not present in sufficient concentration to give a microscopically visible reaction.

In further support of this view it may be recited that vitreous humor, which presents a web-like coagulum that reacts positively to the periodic acid Schiff method of McManus, has never stained metachromatically in our hands despite the use

21. Lillie, R. D., and Mowry, R. S.: Histochemical Studies on Absorption of Iron by Tissue Sections, Bull. Internat. A. M. Mus. **30**:91, 1949.

of a wide variety of acid and neutral, aqueous, alcoholic and other nonaqueous fixatives. Hotchkiss [22] listed hyaluronic acid among the substances giving a strongly positive spot test by the periodic acid Schiff method.

A further corollary of these observations would be that streptococcus hyaluronidase must also contain a chondromucinase, since it, too, destroys the metachromatic staining of umbilical cord matrix and arterial mucins. Indeed, if exposure is sufficiently prolonged, metachromatic staining of cartilage matrix is distinctly impaired after digestion with this enzyme.[23] Boiling in acetic acid impairs considerably the power of this enzyme to remove the metachromatically staining substances from umbilical cord but does not completely destroy it.

CONCLUSIONS

Highly concentrated bovine testicular hyaluronidase contains a thermolabile enzyme, hyaluronidase, which depolymerizes hyaluronic acid in vitro, and a chondromucinase, which, while thermolabile at pH 5.5 and 6.5, resists boiling at pH 3.

Testicular chondromucinase, when thus purified, still destroys all of the metachromatically staining materials of umbilical cord matrix and arteries and of the ground substance of cartilage. Preparations containing the thermolabile hyaluronidase as well have no additional demonstrable effect on this staining reaction. The ferric ion absorption technics of Hale and of Lillie and Mowry also fail to distinguish between the effects of raw and acid-boiled testicular hyaluronidases, and hence probably demonstrate chondromucin alone, rather than hyaluronic acid.

22. Hotchkiss, R. D.: Microchemical Reaction Resulting in Staining of Polysaccharide Structures in Fixed Tissue Preparations, Arch. Biochem. **16**:131, 1948.

23. Lot H 57-4, presented by Wyeth, Inc., was used.

DYNAMICS OF INFLAMMATION AND OF REPAIR

I. The Trigger Mechanism of Acute Inflammation

VIRGIL H. MOON, M.D.
AND
GEORGE A. TERSHAKOVEC, M.D.
CORAL GABLES, FLA.

MANY factors in acute inflammation are well understood. These include the sequences of vascular and cellular changes which appear immediately after injury to living tissues. It is generally agreed that these changes result from any type of injury; also, that the reactions are defensive in nature, tending to limit the spread of the injurious agent, to neutralize or destroy it, and to facilitate repair. There is not yet complete agreement regarding the mechanism by which acute hyperemia, capillary permeability, and leukocytic infiltration are initiated by damage to cells. Accordingly, an experimental investigation of these phenomena was undertaken.

One current interpretation is based on physiologic principles. The animal organism is provided with sundry defense mechanisms against stress or emergencies, mechanisms which are activated by some factor inherent in the emergency itself. It was Pflüger's dictum that the condition giving rise to a need likewise provides the means for supplying the need. This principle seems to apply to the defensive reactions of living tissues that have been submitted to injury. Ebbecké [1] made experimental studies on the effects of simple injuries of human skin. He noted that the first effect was temporary blanching of the skin, followed immediately by hyperemia. Then the center of the hyperemic area became swollen and pale as acute edema developed in the injured skin. This reaction was exemplified in the phenomena of dermographia which follow mild mechanical injury to the skin of susceptible persons. Ebbecké found that other forms of injury—thermal, electrical, or chemical—produced similar effects. He concluded that irritational and inflammatory hyperemia do not result from the effect of nerve stimuli but from the effect of substances released locally by injured cells.

The studies of Lewis [2] on local irritation of human skin corroborated and substantiated those of Ebbecké. The responses to various forms of local injury had similar characteristics regardless of the type of injury. The reactions consisted of (1) local dilatation and hyperemia of the minute vessels, (2) a spreading flare due

From the Department of Pathology, University of Miami Medical Research Unit.

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1. Ebbecké, U.: Die lokale vasomotorische Reaktion der Haut und der inneren Organen, Arch. ges. Physiol. **169**:1, 1917; Über Gewebsreizung und Gefässreaktion, ibid. **199**:197, 1923.

2. Lewis, T.: Blood Vessels of the Human Skin and Their Responses, London, Shaw & Sons, Ltd., 1927.

to reflex dilatation of the adjacent arterioles, (3) a pale, circumscribed wheal due to edema resulting from increased permeability of the endothelium. This "triple response" was made to various agents, including histamine, burning, freezing, electrical current, intense light, various chemicals, mustard oil, cantharidin, bites and stings of insects, peptone, and foreign proteins in sensitized subjects. The same response lacking the spreading flare occurred also in denervated areas of skin; hence it was not due to nerve impulses. Lewis concluded that injured cells liberate an unidentified substance which he called "H-substance" because its action resembled that of histamine. H-substance was defined as a substance or substances producing a local reaction like that of histamine.

Krogh [3] corroborated the interpretation that some substance is liberated from the skin of mammals whenever and however the cells are injured and that it affects the capillaries in two particulars: It causes them to dilate, producing local hyperemia, and it increases their permeability, causing local edema and swelling. In these particulars its effects resemble those of histamine. Krogh believed that H-substance is a normal metabolite of cells, as claimed by Lewis.

The evidence cited indicates that the initial features of inflammation, i. e., hyperemia, endothelial permeability, and edema, result from substances released by damaged cells. But the authors adduced no evidence regarding the phenomenon of leukocytes migrating into the affected tissue, which is a most important item in the inflammatory reaction. Moon [4] made local burns (water at 90 C.) on human and animal skin under conditions of surgical asepsis. Leukocytes promptly migrated into the injured tissue, indicating a chemotactic effect. Since no infection and no foreign substance had been introduced, he attributed this effect to some substance set free by the damaged tissue cells. Subsequently, Silverman,[5] using the technique of McCutcheon and Dixon,[6] demonstrated that cells of normal human skin have a chemotactic index comparable to that of various pathogenic bacteria. Rigdon [7] showed that aqueous extracts of normal tissues, intradermally injected, caused capillary permeability followed by acute inflammation. The permeability was demonstrated visually by the localization of trypan blue injected intravenously. Histologic examination showed large numbers of polymorphonuclear cells in the lesions when no dye had been injected.

The evidence as summarized supports the interpretation that the cardinal features of acute inflammation result from the action of some substance or substances released locally by damaged cells. Another interpretation explains the essential features of acute inflammation as the effects of leukotaxine. Menkin [8] made an extensive series of chemical studies on inflammatory exudates. He subjected purulent exudates, obtained from suppurative pleuritis, to chemical cleavage. By fractionation and purification he obtained a crystalline substance which he termed

3. Krogh, A.: Anatomy and Physiology of the Capillaries, ed. 2, New Haven, Conn., Yale University Press, 1929.

4. Moon, V. H.: Mechanism of Acute Inflammation, Arch. Path. **20**:561 (Oct.) 1935.

5. Silverman, D.: A Chemotropic Substance Derived from Normal Tissues, Arch. Path. **25**:40 (Jan.) 1938.

6. McCutcheon, M., and Dixon, H. M.: Chemotropic Reactions of Polymorphonuclear Leukocytes to Various Micro-Organisms, Arch. Path. **21**:749 (June) 1936.

7. Rigdon, R. H.: Demonstration of Capillary Permeability Factor in Tissue Extracts from Normal Rabbits, Arch. Surg. **41**:96 (July) 1940.

8. Menkin, V.: The Dynamics of Inflammation, New York, The Macmillan Company, 1940.

leukotaxine. This substance was shown to produce capillary dilatation and permeability, resulting in edema, and to have strong chemotactic properties. On the basis of these studies he postulated that acute inflammation is due to the leukotaxine which is present in the damaged tissues. In order to be convincing, this hypothesis should be supported by showing the presence of leukotaxine in normal tissues before injury of any kind has been done.

An extensive series of local inflammatory lesions was made and studied histologically for additional evidence regarding the mechanism which initiates acute inflammation. The agents employed were aqueous extracts of normal tissues, burns, freezing, turpentine injected into the skin, and albumin injected into the skin of sensitized animals.

METHODS

Tissue Extracts.—A quantity of muscle was excised from a freshly killed rabbit. This was finely ground through a meat chopper, suspended in four times its volume of isotonic sodium chloride solution, and then beaten into a fine suspension by means of an electric disintegrator (Waring blendor®). After centrifugation, the reaction of the fluid was adjusted to pH 7; then the fluid was sterilized by filtration (Seitz), proved sterile by culture and stored under refrigeration until used. Extracts of kidney, liver, skin, and lung were prepared in the same manner.

Small quantities (0.02 to 0.04 ml.) were injected intradermally into the ears of rabbits with a 28-gage needle. From four to six such injections were made at intervals in the same rabbit. These injections provided lesions ranging in duration from 15 minutes to 24 hours in the same animal. Controls consisted of 0.04 ml. of isotonic sodium chloride solution similarly injected.

Thirty-three such injections of muscle extract were made, 23 of kidney extract, 10 of extract of skin, 15 of extract of liver, and 15 of extract of lung.

Turpentine.—A 10% solution of turpentine N. F. was made, sesame oil being used as a diluent. Injections of 0.02 ml. were made intradermally with a 28-gage needle. Similar quantities of sesame oil alone were injected as controls. Nine such injections were made in rabbits, eight in guinea pigs, and six in monkeys.

Thermal Injuries.—Burns were made by filling a test tube, 8 mm. in diameter, with water at 90 C. and applying it to the skins of animals for 10 seconds. Ten such burns were made in rabbits and 55 in guinea pigs. No controls seemed appropriate to this experiment.

Local lesions about 6 mm. in diameter were made by applying a pencil of solidified carbon dioxide to the skin for a period of 30 seconds. Twelve such lesions were made in rabbits and 60 in guinea pigs.

Allergic Inflammation.—Guinea pigs weighing about 400 gm. were sensitized by injecting 0.1 ml. of a 1% solution of crystalline egg albumin subcutaneously or intraperitoneally. After periods of 12 to 14 days, 0.02 ml. of a 1% solution of albumin was injected intradermally with a 28-gage needle. Such injections were made in series in the same animal, thus providing lesions ranging in duration from 15 minutes to 24 hours. A total of 58 such lesions were made.

In all instances the animals were killed painlessly and preparations of the lesions made for histologic examination.

RESULTS

Tissue Extracts.—Uniformly, extracts of normal tissues provoked a prompt inflammatory response when injected into the skin. Hyperemia was visible soon, and the areas became swollen. Histologic examinations revealed hyperemia and edema 15 minutes after injection. The capillaries and venules were packed with leukocytes, which were beginning to migrate into the tissues. Hyperemia, edema, and infiltration increased progressively, reaching a maximum at about 6 hours. There were acute edema, capillary hemorrhages, diapedesis of red cells, and a mesh work of

fibrin infiltrated with many leukocytes (Fig. 1). The latter were chiefly neutrophils, with small numbers of monocytes and lymphocytes. Twenty-four hours after injection the reaction seemed to be regressing; the hyperemia and edema were less marked and monocytes were relatively more numerous than in lesions of shorter duration.

Most of the control injections of isotonic sodium chloride solution caused no reactions. In some instances, hyperemia and edema were visible, and a minimal migration of leukocytes occurred, but no fibrin, capillary hemorrhage, or diapedesis was seen. These lesions subsided rapidly and were not recognizable after 24 hours. It is possible that the distention with injected fluid caused mechanical injury to the tissues sufficient to produce a minor reaction.

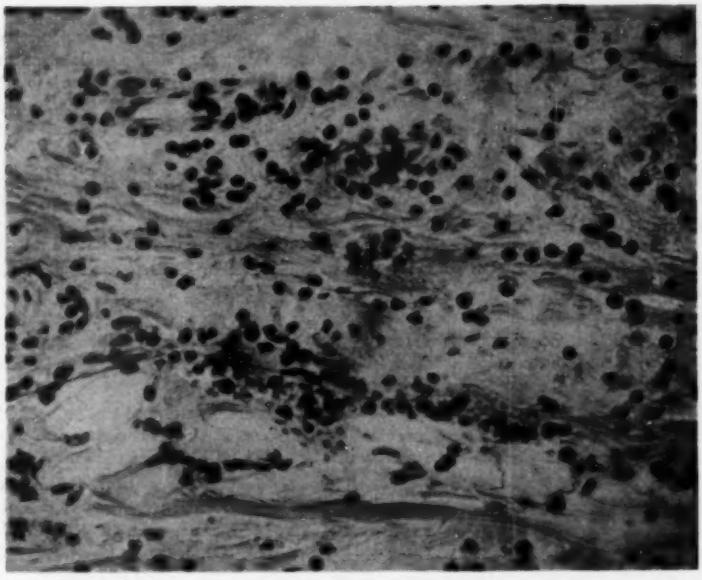


Fig. 1.—Acute inflammation, with capillary hemorrhage, four hours after injection of muscle extract (× 280).

The sesame oil, used as a control on the effects of turpentine, regularly caused a mild reaction—more transient and less intense than that of turpentine. It is well known that tissues are irritated by the presence of a foreign substance even if that substance is bland in character. That such a reaction was present in the control tests does not invalidate, but rather corroborates, the thesis that injury or irritation of any kind results in an inflammatory response.

Thermal Injuries.—Several types of thermal injuries were made for both systemic and local effects. In the former, the fur on the back, shoulders and hips was clipped closely to decrease its insulating effect. The rabbits were then completely anesthetized with ether, and the areas mentioned were immersed in water at 90 C.

for five seconds. Subsequently the animals were given morphine hypodermically to prevent pain. Freezing was done under the same conditions by dipping the areas mentioned into a mixture of alcohol and solidified carbon dioxide for 30 to 60 seconds. The temperature of the freezing mixture was —20 to —30 C.

These experiments were made primarily to secure data on systemic leukocytosis, which will be reported later. After varying intervals, the animals were killed painlessly and sections of skin and subcutaneous tissue were examined microscopically. Massive edema was present, but hyperemia was not conspicuous. The zone of subcutaneous tissue adjacent to the burn was densely infiltrated with leukocytes. It appeared that these were strongly attracted to the superficial necrotic tissue of the burn (Fig. 2).

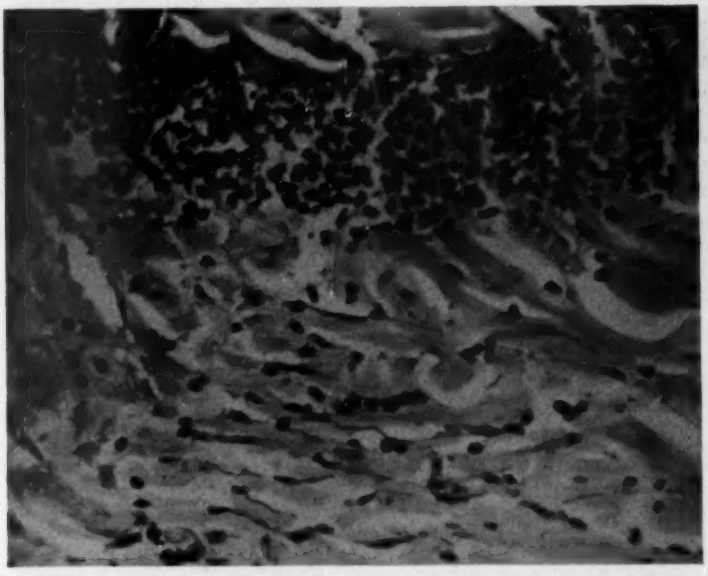


Fig. 2.—Subcutaneous tissue, dorsal area, 48 hours after a superficial burn. Severe edema with leukocytes strongly attracted to the necrotic tissue, shown at the top (× 280).

Local burns were made as described under "Methods." A rather marked difference was seen between the degree of inflammatory reaction in large areas subjected to scalding or freezing and that seen in the small local lesions. A possible reason for this difference is that the small lesions were made on the ears, which have a more abundant blood supply than has the skin over the body. There was massive edema of the tissues in scalded areas, but other features of inflammation were less severe than those seen in the small lesions made on the ears.

Small local lesions were induced on areas of the abdominal skin of guinea pigs by scalding and freezing. These resulted in prompt acute inflammatory changes characterized by hyperemia, edema, capillary hemorrhages, diapedesis, fibrin

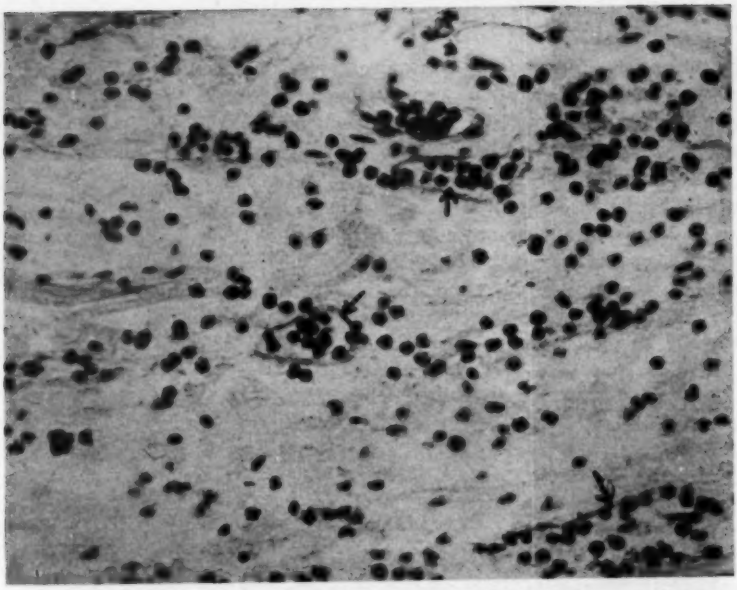


Fig. 3.—Subcutaneous tissue, dorsal area, four hours after freezing. Edema is conspicuous. Leukocytes are numerous within the venules and capillaries (arrows) (× 280).

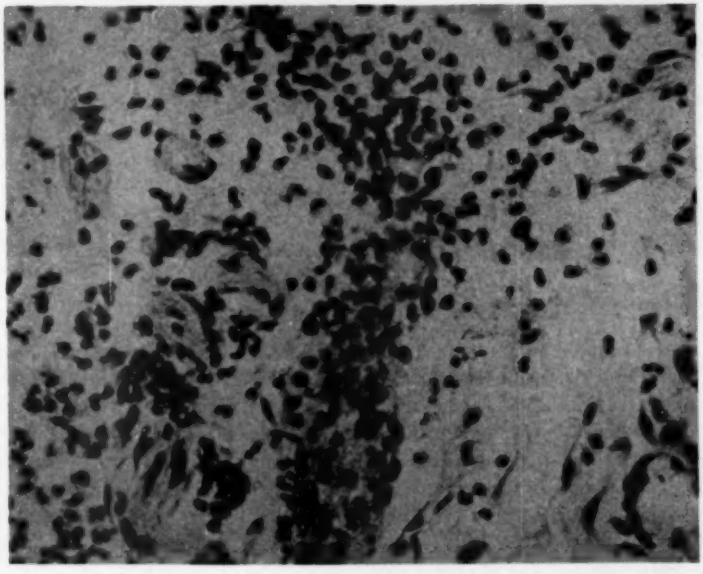


Fig. 4.—Allergic inflammation 20 minutes after albumin was injected into the skin of a sensitized guinea pig (× 280).

deposits, and leukocytosis. These changes were seen after both scalding and freezing but were more mild in the lesions caused by the latter injury (Fig. 3). Exactly similar reactions followed both scalding and freezing on the ears of rabbits.

Anaphylactic Injury.—A total of 58 local anaphylactic lesions were made in the skin of sensitized guinea pigs, as described under "Methods." Regularly an immediate inflammatory reaction developed. An area approximately 1 cm. in diameter surrounding the point of injection became reddened, then pale and swollen, producing a wheal like that seen in human subjects inoculated with a substance to which they are sensitive. Microscopic examinations showed acute hyperemia, edema, and frequently capillary hemorrhages in the cutaneous and subcutaneous tissues. Neutrophils actively infiltrated the areas within 20 minutes (Fig. 4). Edema, capillary hemorrhages, diapedesis of red cells, and leukocytic infiltration increased progressively, reaching their maximum in about six hours. A deposit of fibrin was usually visible in these areas (Fig. 5). In many instances there was necrosis of the epithelium and of the underlying muscular fibers.

Fig. 5.—Allergic inflammation four hours after albumin was injected into the skin of a sensitized guinea pig. Hyperemia, diapedesis, edema, and acute neutrophilic infiltration are shown (× 280).

It is generally accepted that inflammation results when antibody and antigen meet within living tissue (Opie [9] has reviewed the literature). This is equivalent to stating that cells are injured when antibody and antigen meet within them. The degree of that injury often is severe, resulting in necrosis, as was seen in many of our sections.

9. Opie, E. L.: Inflammation and Immunity, J. Immunol. **17**:329, 1929.

COMMENT

In these experiments the development of reactions to various forms of simple injury uncomplicated by infections was studied. The observation that the agents used will induce inflammation is not new; many other agents will do likewise. Three hundred twelve such lesions were examined histologically, not including controls.

We regard the results from injections of tissue extracts as particularly significant. Efforts had been made to minimize autolytic and chemical changes so that the extracts might contain unchanged cellular substances in solution. The first response was vascular, consisting of hyperemia and edema, indicating increased endothelial permeability. In these particulars the extracts might be said to contain H-substances. As shown by controls, these effects were not due to the saline solution, nor to the associated mechanical trauma.

Migration of leukocytes was evident within 30 minutes. Probably these were attracted by substances in the extracts injected. Had this effect been due to infection, it should have been equally prominent in the controls. Aqueous extracts of normal tissues are strongly chemotactic, as shown by several hundred tests which we have made (data to be published). Hence it is suggested that such extracts may cause dilatation of vessels, increased endothelial permeability, and chemotaxis.

In the case of thermal injuries no foreign matter of any kind was implicated; the reaction must have been evoked by substances released from the damaged tissues. The most immediate and intense responses were found in anaphylactic reactions. Minute amounts of albumin injected into *normal* guinea pigs caused no more effect than did saline solution. Hence we believe that allergic damage to cells evoked the same vascular and cellular phenomena as did other forms of injury.

It is our impression that the tissue substance which produced the effects mentioned is not a specific chemical entity, although this belief is not based on chemical evidence. It seems more probable that the active agent is merely the cytoplasmic substance of the damaged cells. Injury may affect the integrity of the cell wall, allowing the escape of cytoplasmic substance into the tissue spaces as suggested by earlier reports. Cutaneous injury affects several types of tissue, including epithelium, fibrous tissue, endothelium, and muscle. The unaltered cytoplasmic substances of these tissues probably are not chemically identical. It was Krogh's belief that the H-substance, which evokes the vascular response to injury, is not identical in the various types of tissue.

Our observations do not support the supposition that leukotaxine plays a part in initiating inflammation. Thus far, leukotaxine has been demonstrated only in inflammatory exudates. No trace of any such exudate was found in the normal tissues adjacent to the lesions we produced. Those lesions developed spontaneously immediately after normal tissues were injured. Extracts of normal tissues caused each of the reactions characteristic of leukotaxine, i. e., hyperemia, endothelial permeability, and leukocytic infiltration. Perhaps a substance having the properties of leukotaxine might be isolated from normal tissues; if so, the term "cytotaxine" would be a more logical designation. Others have been content to speak of this merely as H-substance.

The conclusion that released cytoplasmic material evokes the inflammatory response is consonant with the conclusions of Ebbecké, Lewis, Krogh, and other

earlier workers. This is the only interpretation supported by each feature of our experiments. A physiologic provision whereby injured cells may issue their own call for help and activate the mechanisms of defense is admirably adapted to the purposes which inflammation serves. "The perfection of this mechanism is such that the defense is organized immediately and at every threatened point; it is arranged and carried through locally, being independent of higher systems of control (nervous) and of distribution (cardio-vascular)."—Lewis.

CONCLUSIONS

Earlier workers showed, by evidence which has not been questioned, that local hyperemia and edema result promptly from the effects of substances released by damaged cells. Our results support that interpretation.

The experiments reported here indicate that substances derived from damaged cells also attract leukocytes into the area, thus completing the picture of beginning inflammation. Such substances appear to serve as the trigger mechanism by which forces are activated for local defense.

MORPHOLOGICAL CHANGES OF ADRENAL CORTEX IN FROSTBITTEN RABBITS WITH AND WITHOUT HEPARIN TREATMENT

BERNHARD HOELSCHER, M.D.
RANDOLPH FIELD, TEXAS

IN SEVERAL papers [1] published in recent years, attention has been given to experimental frostbite from the standpoint of functional pathology and to preventing tissue damage after exposure to local cold. Furthermore, investigations have been made to determine how cold affects the adrenal gland. Selye [2] (1946) and associates, and later other investigators, have shown that, like other forms of stress, cold evokes stimulation of the pituitary-adrenal system. Selye termed this phenomenon the "general adaptation syndrome."

In the development of this theory, the most widely employed form of stress has been that of exposing animals to general cold. There is still little information concerning the morphological changes that occur in the adrenal glands of animals exposed to local cold. The purpose of this investigation was to study the adrenal cortex histologically after the application of this type of stress. Since the clinical picture in animals exposed to local cold depended on the severity of the cold injury, it was deemed advisable to determine the degree of adrenal change in relation to the severity of frostbite. It seemed desirable also to observe the adrenal changes in animals exposed to local cold and subsequently treated with heparin, since Lange and Boyd,[1a] (1945) and Lange and Loewe [1c] (1946) have reported beneficial results from heparin therapy in frostbite.

MATERIAL AND METHODS

The experiments were performed on white male rabbits, since female animals seem to be more susceptible than males to internal hemorrhage after heparin administration. The animals weighed over 2,000 gm. and were kept in the open air throughout the year. At least one week before the experiments they were brought into the air-conditioned animal room and kept at + 25 C. They were fed purina® rabbit pellets ad libitum and occasionally carrots. They had free access to water. The method used in the first part of the experiments corresponded as closely as possible to the procedure of Lange and associates except that the animals were not anesthetized during freezing. The right hindleg was immersed to the knee joint in a bath consisting

From the Department of Pathology USAF School of Aviation Medicine, Randolph Air Force Base.

1. (*a*) Lange, K., and Boyd, L. J.: Surg., Gynec. & Obst. **80**:346, 1945. (*b*) Lange, K.; Boyd, L. J., and Loewe, L.: Science **102**:151, 1945. (*c*) Lange, K., and Loewe, L.: Surg., Gynec. & Obst. **82**:256, 1946. (*d*) Lange, K.; Weiner, D., and Boyd, L. J.: New England J. Med. **237**:383, 1947. (*e*) Friedman, N. B.; Lange, K., and Weiner, D.: Am. J. M. Sc. **213**:61, 1947. (*f*) Fuhrman, F. A., and Crismon, J. M.: J. Clin. Invest. **27**:364, 1948. (*g*) Quintanilla, R.; Krusen, F. H., and Essey, H. E.: Am. J. Physiol. **149**:149, 1947.

2. Selye, H.: J. Clin. Endocrinol. **6**:117, 1946.

of 95% alcohol cooled with solid carbon dioxide (dry ice). Pichotka and Lewis [3] (1949) have described this procedure in detail. As the experiments progressed, the evaluation of heparin therapy necessitated use of a less severe cold injury.

The experimental groups were as follows:

Group A: Controls (not exposed, untreated).
Group B.: Not exposed, heparin-treated.
Group C.: Exposed to — 15 C. for 30 min., untreated.
Group D.: Exposed to — 15 C. for 30 min., heparin-treated.
Group E.: Exposed to — 15 C. for 30 min.; frostbitten legs immediately rewarmed in 42-C. water for five minutes.
Group F.: Exposed to — 25 C. for 30 min., untreated.
Group G.: Exposed to — 25 C. for 30 min., heparin-treated.

Each of the heparin-treated animals received 3 cc. of heparin sodium solution [4] intravenously every six hours for six days.

For the morphological studies, adrenal glands were taken only from those animals killed by guillotine to exclude the influence of postmortem changes. Likewise, animals with internal hemorrhages were not considered. All together, 199 animals were evaluated under the conditions mentioned. The statistical method used was a test of significance using the analysis of covariance.

Summary of Experimental Data

Group and Number	Temperature and Exposure Time	Treatment	Experimental Time, Days	Av. Body Wt., Gm.		Av. Change in Body Wt. During Experiment, Gm.	Av. Adrenal Wt., Mg.	Relative Adrenal Wt., Mg. per 100 Gm. Body Wt.	P*
				Initial	Final				
A (28)	None	None	0	.....	2,693	Not determined	174	6	....
B (36)	None	Heparin	6	2,610	2,458	—152	206	8	.030
C (31)	—15 C. for 30 min.	None	6-10	3,061	2,879	—181	278	10	<.001
D (23)	—15 C. for 30 min.	Heparin	6	2,319	2,153	—166	208	10	.007
E (41)	—15 C. for 30 min. rapidly rewarmed	None	6-8	2,704	2,638	— 66	269	10	<.001
F (20)	—25 C. for 30 min.	None	6-10	2,940	2,488	—452	287	12	<.001
G (20)	—25 C. for 30 min.	Heparin	6-10	2,696	2,310	—386	256	13	<.001

* Probability levels for statistical tests of significance of adrenal weights for indicated treatment groups against controls (A). In addition, it was found that there was no significant difference between Groups C and E ($P > 0.50$).

Immediately after the animal was killed, the adrenals were freed from adherent fat tissue, weighed, and fixed with Susa fluid and 10% formaldehyde solution. The adrenals fixed with Susa fluid were embedded in paraffin and stained with hematoxylin and eosin. The formaldehyde-fixed organs were cut with a freezing microtome and stained with Sudan III. The experiments were performed during winter and summer months.

RESULTS

The clinical results of heparin treatment in this series were reported in detail by Pichotka and Lewis [3] (1949). In short, the rabbits did not show any benefit from heparin treatment so far as the extent and the degree of necrosis of the exposed leg were concerned. Furthermore, the death rate of the heparinized animals was greater than that of the nonheparinized frostbitten animals. Some heparin-treated animals suffered acute shock and died after the first or the second injection. In other heparinized animals severe bleeding into the peritoneal cavity and retro-

3. Pichotka, J., and Lewis, R. B.: Proc. Soc. Exper. Biol. & Med. **72**:127, 1949.

4. The heparin used was solution liquaemin ® sodium (Organon, Inc., Orange, N. J.) and solution heparin sodium (Abbott Laboratories, North Chicago, Ill.).

peritoneal space was observed; these hemorrhages were usually so severe as to cause death. Fatal hemorrhages occurred more frequently during the winter months.

As shown in the table, a drop in body weight was generally found in the frostbitten animals whether untreated, treated with heparin, or rewarmed. Weight loss was found also in normal animals given heparin. Conversely, a statistically significant increase in the relative adrenal weight resulted in all frostbitten animals. The relative adrenal weight was increased also in heparinized animals not exposed to cold. However, the difference between Groups C and E (table) was not significant, indicating that the increase in adrenal weight was about the same in rapidly rewarmed animals and those thawed at room temperature. Exposure to —25 C. evoked the greatest increase in the relative adrenal weight, particularly in those animals additionally treated with heparin.

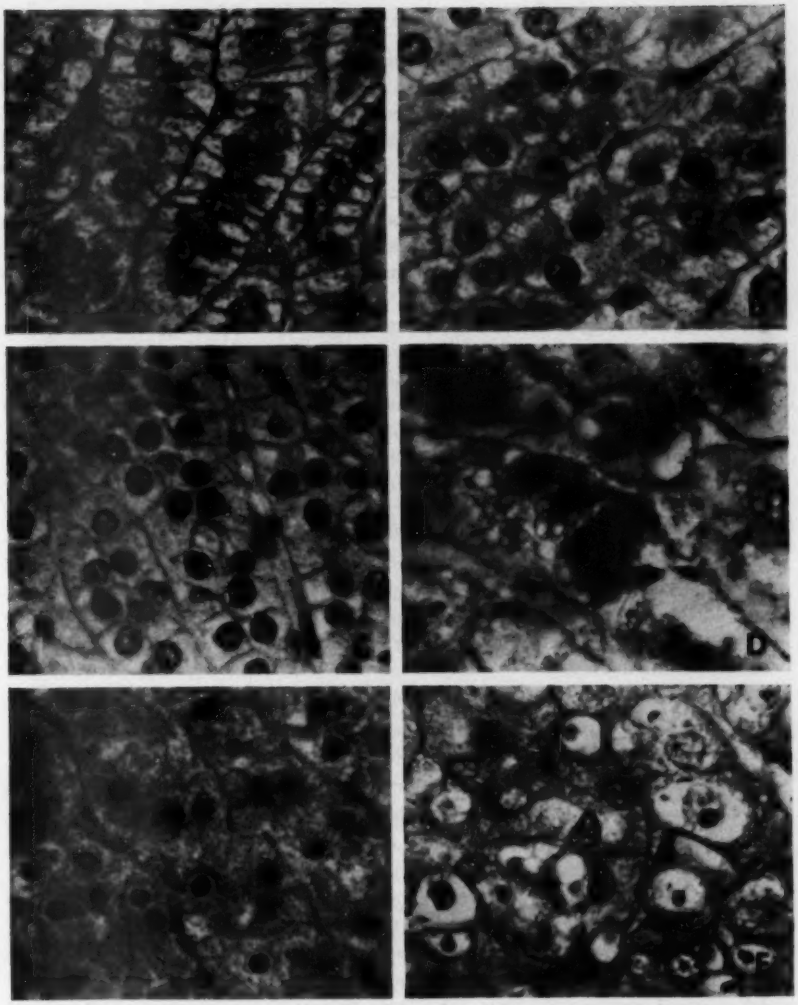
The well-known appearance of an adrenal gland of an untreated rabbit is given in the figure for a comparison of the results.

The histological observations of the adrenal glands for different series of animals were as follows:

Heparin Controls.—There was a distinct seasonal difference in the histological changes caused by heparin. In the group of animals treated with heparin during the summer months, only an enlargement of the cortical cells was found. Practically throughout the entire gland the nuclei appeared enlarged and vesicular. The visible chromatin substance was small in amount. The cytoplasmic structure of these cells did not show great differences as compared with that of cortical cells of untreated animals except that, as a rule, the cell borders were not sharply defined. On the other hand, the adrenal glands of animals treated with heparin during the winter months showed comparatively distinct histological changes. Generally, a definite enlargement of these cells was observed, with large vesicular nuclei of the cells in the fascicular layer (*B* in figure). Some cells were found, in addition, to be severely affected. These cells showed an appearance of nuclear disintegration associated with coagulation of the cytoplasm, which had shrunk to an eosinophilic mass, or occasionally to several small homogenized cytoplasmic fragments scattered throughout the cells and unstained areas in the cytoplasm.

Frostbitten Animals Either Spontaneously Rewarmed at Room Temperature or Rapidly Rewarmed in 42-C. Water.—The cells of the adrenal glands, especially those of the zona fasciculata, appeared swollen (*C* in figure). Cloudiness of the cytoplasm with poor demarcation of cell borders was seen at times, and nuclear pyknosis was found occasionally. The endothelial cells of the sinusoids were more distinctly visible than in untreated animals, probably because of concomitant swelling. Often a disarrangement within the cell columns of the fascicular layer was produced by the enlargement of the affected cells. The adrenal glands of the rapidly rewarmed animals did not show any essential difference from those of the animals rewarmed at room temperature.

Frostbitten and Heparinized Animals.—Especially interesting were the histological observations on the adrenal glands of the animals exposed to cold and immediately heparinized. In these adrenal glands all stages of necrosis of the cortex were observed, being most pronounced in those animals which were exposed to —25 C.



All photomicrographs are shown at a magnification of × 738.

A, zona fasciculata of the adrenal cortex of a normal rabbit. Hematoxylin and eosin.

B, adrenal cortex of a rabbit heparinized every six hours for six days. The zona fasciculata shows large, vesicular nuclei. Some nuclei appear pyknotic. Hematoxylin and eosin.

C, adrenal cortex of a frostbitten rabbit. The animal had been exposed to —15 C. for 30 min. There is disarrangement within the cell columns of the zona fasciculata. The nuclei are enlarged; the cell borders, not distinct.

D, adrenal cortex of a rabbit exposed to —25 C. for 30 min. and immediately heparinized every six hours for six days. The zona fasciculata shows a honeycomb-like appearance of the cytoplasm and clusters of pyknotic nuclei. Hematoxylin and eosin.

E, adrenal cortex of a rabbit exposed to —15 C. for 30 min. and heparinized every six hours for six days. The zona fasciculata shows nuclei with pyknotic centers surrounded by ring-shaped clear areas. Hematoxylin and eosin.

F, adrenal cortex of a rabbit exposed to —25 C. for 30 min. and immediately heparinized every six hours for six days. Cells of the zona fasciculata show the cytoplasm coagulated to a homogeneous mass, with nucleus pyknotic, surrounded by a clear, colorless area. Hematoxylin and eosin.

In some sections an appearance of vacuolar degeneration of the epithelial cells of the zona fasciculata was present (*D* in figure). A foamy or honeycomb-like appearance of the cytoplasm was observed. The fine walls of the honeycomb were acidophilic. In both frozen and paraffin sections the vacuolated areas appeared optically to be empty. Their contents did not respond to fat stains. The nuclei were deformed and pyknotic. Sometimes clusters of such pyknotic nuclei were seen.

In other sections the cells showed the cytoplasm coagulated into more or less coarse granules. The nuclei of these cells demonstrated pyknotic centers surrounded by light ring-shaped areas between the nuclear membranes and the pyknotic centers (*E* in figure).

Finally, cell changes, with the cytoplasm coagulated to a homogenous eosinophilic mass and nuclei pyknotic, were observed. Often the pyknotic nucleus and the shrunken cytoplasm formed a homogenous mass surrounded by a clear, colorless area (*F* in figure). The typical arrangement of the cortical cells in different layers of the normal adrenal cortex did not exist. The sinusoids were distinctly dilated, and the capillary walls showed swollen endothelial cells. There were no signs of connective-tissue replacement. The frozen sections were mostly devoid of fat.

COMMENT

The above-mentioned histological results show that heparin therapy administered for frostbite during the winter months caused severe adrenal damage. The changes observed during the summer months were less pronounced. Pichotka and Lewis [3] observed a greater frequency of fatal internal hemorrhages in heparinized rabbits in winter than in summer.

The following two points seem worthy of more critical discussion:

1. The possibility that the histological changes and the gain in weight of the adrenal cortex may be related to Selye's concept of the general-adaptation syndrome.

2. The possibility that heparin treatment may augment the degenerative changes of the cortical tissue observed in frostbite.

As clearly shown by Pichotka and Lewis,[3] the degree and the extent of local cold injury were strikingly related to the ambient bath temperature at the constant exposure time of 30 min. The lower the temperature, the greater were the incidence and the extent of subsequent gangrene. Consequently, the breakdown products of the tissue damaged by frostbite must be assumed to have been increased as the degree of injury became more severe. Among the metabolites liberated from traumatized tissue, histamine has been considered the most important, since Abel and Kubota [5] (1919) developed the theory that it is discharged from injured tissue and is the fundamental cause of wound shock. Shimada [6] (1935) reported studies dealing with a specific toxic substance occurring in frozen tissue. Whatever the nature of these histotoxic metabolites may be, they represent substances stressing the affected organism. As Selye has pointed out, these toxic metabolites cause the hypophysis to release adrenocorticotropic hormone, which in turn stimulates the adrenal cortex to form an excess of cortical hormones, provided the damage is not severe enough to cause death.

5. Abel, J. J., and Kubota, S.: J. Pharmacol. & Exper. Therap. **13**:243, 1919.

6. Shimada, T.: Mitt. med. Gesellsch. zu Tokyo **49**:1130, 1935.

So far as the time of adaptation is concerned, the adrenal glands described in this paper would appear to belong to the resistance phase and the exhaustion phase of the general adaptation syndrome (Selye,[2] 1946). As the amount of gangrenous tissue became greater, the weight of the reacting adrenal cortex increased. The morphological degenerative changes of the cortical cells, being particularly severe in frostbitten, heparinized animals, correspond to the stage of exhaustion indicated by the serious clinical appearance of those animals before being killed. Furthermore, the adrenal glands of the latter animals were almost devoid of fat in frozen sections.

It should be mentioned that in determining the rectal temperature of animals while they were exposed to local cold a drop in body temperature of 1 C. to 2 C. was generally found in most of them. It seems unlikely that this decrease in body temperature is essential for the production of the severe adrenal changes observed.

For the increased incidence of degenerative changes observed in cortical tissues of frostbitten and heparinized animals, in contrast to the nonheparinized, one explanation seems reasonable. In the areas exposed to cold there occurs an increase in the permeability of the capillary walls because of the toxic metabolites liberated from the damaged tissue (Lewis,[7] 1927). These poisonous breakdown products travel from the cold-injured part through the damaged capillaries into the blood stream and affect the entire organism. Under the influence of these noxious agents plasma migrates into the intercellular spaces, with subsequent hemoconcentration, as the result of the increased capillary permeability, which may lead to shock. Rössle[8] (1943) and his co-workers found this picture of plasmic transudation in shock of different origins and concluded that transudate between capillary wall and parenchyma impedes oxygen diffusion, which results in hypoxia and degeneration of the parenchyma. Under heparin treatment the plasma presumably is able to escape much more easily from the blood stream through the endothelial membrane, particularly if it is hyperpermeable. The adrenal gland, as the site of increased activity in cases of stress, must be considered highly susceptible to such an increased transudation in frostbite. It readily fails and finally undergoes degeneration, such as that observed in heparinized frostbitten animals.

SUMMARY AND CONCLUSIONS

A total of 199 adrenal glands from frostbitten rabbits, with and without heparinization, were investigated histologically. Only those adrenal glands fixed immediately after autopsy were evaluated.

The increase found in the adrenal weight was in direct relation to the degree of cold injury and was accompanied by a decrease in body weight.

In animals that received heparin but were not exposed to cold the adrenal weight was slightly augmented.

In severe cold injury degenerative changes of the adrenal cortex were observed, which were increased in severity by heparin treatment.

7. Lewis, T.: The Blood Vessels of the Human Skin and Their Responses, London, Shaw & Sons, Ltd., 1927.

8. Rössle, R.: Virchows Arch. path. Anat. **311**:252, 1943.

Case Reports

ACUTE PLASMA LEUKEMIA WITHOUT LOCALIZED BONE LESIONS

Report of a Case with Autopsy Observations

S. H. LAWRENCE, M.D.
AND
HARRY C. FORTNER, M.D.
SAN FERNANDO, CALIF.

PLASMA-CELL leukemia without circumscribed bone lesions has been only rarely described. The following case is interesting because careful postmortem examination, including x-ray survey, showed no bone defects, although the marrow was replaced by diffuse plasma-cell infiltrate.

CLINICAL HISTORY

E. J. N., a white man, 55, was admitted to the San Fernando Veterans Administration Hospital (Case 11520) on Feb. 20, 1950, because he had been coughing bloody sputum for the preceding three days.

The patient had pulmonary tuberculosis, which was diagnosed in 1934. His disease was said to have become arrested shortly after this, but in 1938 the disease was again found to be active. At this time the x-ray photograph showed a bilateral exudative lesion of the apexes with several small cavities on the left. In 1941 a left pneumothorax was begun, and this was continued until 1945. A pneumothorax was initiated on the right in 1941 and carried for 18 mo., when the space was lost. In 1947 he was hospitalized in a sanatorium for four months and after that his disease was again considered arrested. He entered a Veterans Administration domiciliary home in June, 1947, and remained there until October, 1949. Since then he had been living in a hotel room.

Six weeks prior to admission he began to notice "bruises" appearing on his body, especially over the thighs. He denied injury or medication. Three weeks prior to admission, following slight trauma, nosebleed occurred and was controlled with considerable difficulty. Three days before admission his sputum became bloody, and inasmuch as he knew that this frequently accompanies tuberculosis he sought hospitalization in this institution.

Physical examination revealed a poorly nourished white man, who did not appear particularly ill but whose memory seemed impaired, the history being obtained with some difficulty. He was expectorating small amounts of bloody sputum at the time of examination. Externally the head and neck revealed no abnormality. There was no cervical lymphadenopathy. The larynx was slightly inflamed, and numerous pinpoint to pinhead-size petechiae were seen over the buccal mucosa and palate. There were many petechiae and ecchymotic areas over the entire torso and the extremities. No other abnormalities were found except bronchovesicular breath sounds over the upper half of the right lung field. A capillary-fragility tourniquet test (Rumpel-Leede phenomenon) was positive. The x-ray film showed fibronodular disease, which appeared to be inactive, throughout both upper lung fields. Smears from two 24-hr. sputum concentrates were negative for acid-fast bacilli. The Kahn serologic test for syphilis was negative. The urine

From the Veterans Administration Hospital.

Reviewed in the Veterans Administration and published with the approval of the Acting Chief Medical Director. The statements and conclusions published by the authors are the result of their own studies and do not necessarily reflect the opinion or policy of the Veterans Administration.

contained albumin but no Bence Jones protein. The highest specific gravity found in a Fishberg concentration test of the urine was 1.019, and on this examination there was no albumin. The red-blood-cell count was 4,280,000; the hemoglobin, 14.7 gm. per 100 cc.; the white cell count, 2,700, and the differential count 40% neutrophils, 40% lymphocytes, and 20% eosinophils. The platelet count was 12,800; the bleeding time, 18 min., and the coagulation time, 8 min. The erythrocyte sedimentation rate was 6 mm. per hour.

The patient was afebrile and was given ascorbic acid, menadione, and rutin, and the blood in his sputum gradually diminished. A "buffy coat" white-blood-cell count was made two days after admission. Two hundred cells were counted, with the following results: lymphocytes 15, promyelocytes 6, myelocytes 10, band cells 23, segmented neutrophils 120, eosinophils 5, basophils 1, monocytes 9, plasma cells 7, blast cells 2, and stem cells 2.

Four days after the patient's admission the nurse reported that he did not respond when his breakfast was served. The physician arrived a few minutes later and found him in deep coma. All deep reflexes were hyperactive, but there were no pathological reflexes. The right side of the body was spastic. A lumbar puncture was done, and the fluid was bright red. His condition rapidly became worse, his temperature rising to 105 F. Respirations ceased at 12: 15 a. m.

Postmortem x-ray films of the skull, the long bones, and the pelvis showed no areas of rarefaction.

POSTMORTEM EXAMINATION

The body was that of a well-developed, fairly well-nourished white man. Over chest, abdomen, thighs, and lower extremities there were small subcutaneous greenish and reddish patches—no doubt a type of purpura. There were no palpable superficial lymph nodes. On the surface of the intestines were a very few, scattered purpuric patches.

The liver's edge was at the right costal margin. The mesenteric nodes were only slightly enlarged and were grayish on section, some appearing to contain small hemorrhagic foci. Both pleural cavities were obliterated by dense fibrous adhesions, and it was necessary to remove the parietal pleura with the lungs. No nodes were found in the mediastinum. The pericardial sac contained about 20 cc. of clear amber fluid. On the anterior surface of the heart a few minute subepicardial petechiae were seen.

The sectioned right lung contained a healed fibrotic tuberculous lesion. There was moderate edema, with scattered areas of mild congestion. The middle lobe was rather firm to palpation, and on section showed some hemorrhage involving the parenchyma, with consolidation.

The left lung showed more adhesions, particularly in the apical region. There were scattered yellowish fibrocaseous foci throughout. All tuberculous areas of both lungs seemed healed.

The heart weighed 325 gm. and was essentially normal. The spleen weighed 235 gm. The malpighian bodies were not prominent. The pulp scraped away with moderate ease. The liver weighed 1,560 gm. and seemed normal in appearance.

In the stomach there were quite a few small elevated papillae, one having a definite pedicle. Polypoid structures were found in the large intestine and in the ascending colon, and one located about 7.0 cm. from the ileocecal junction was pedunculated.

The brain weighed 1,580 gm. There appeared to be slight congestion of the vessels, particularly in the anterior portion of the left frontal lobe. Convolutions were flattened and sulci narrowed in this region. Section of the ventricles revealed both to be filled with blood, more on the left than on the right. Serial sections through the cerebrum and cerebellum located a large area of hemorrhage in the

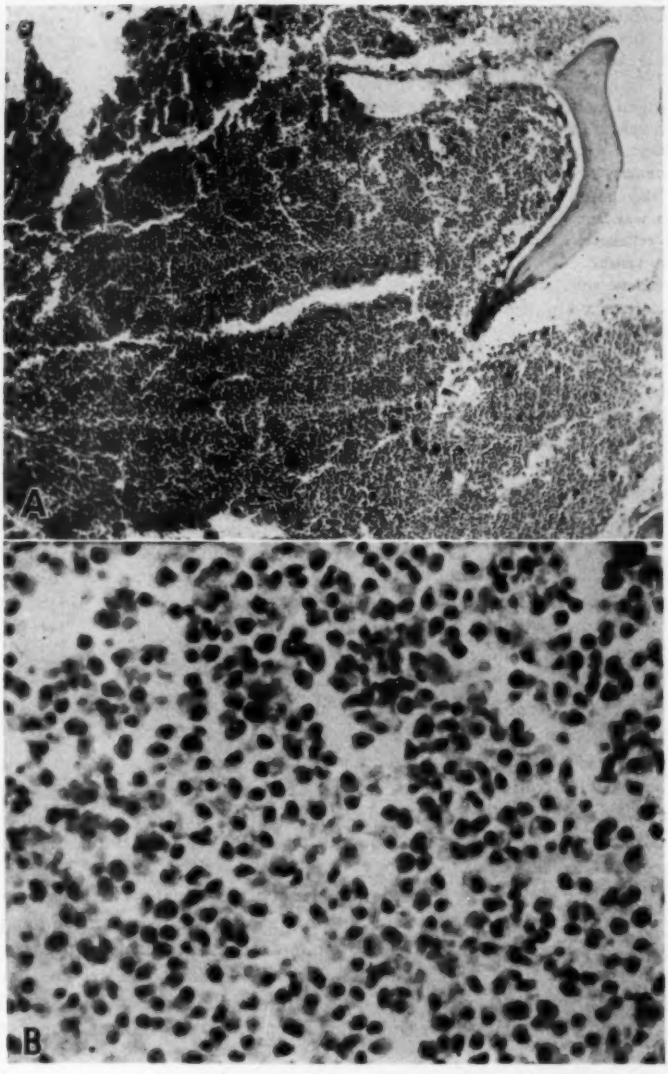


Fig. 1.—*A*, section of bone marrow showing diffuse plasma-cell hyperplasia; hematoxylin and eosin; × 75; Armed Forces Institute of Pathology Accession 189106. *B*, higher magnification of same section; × 395.

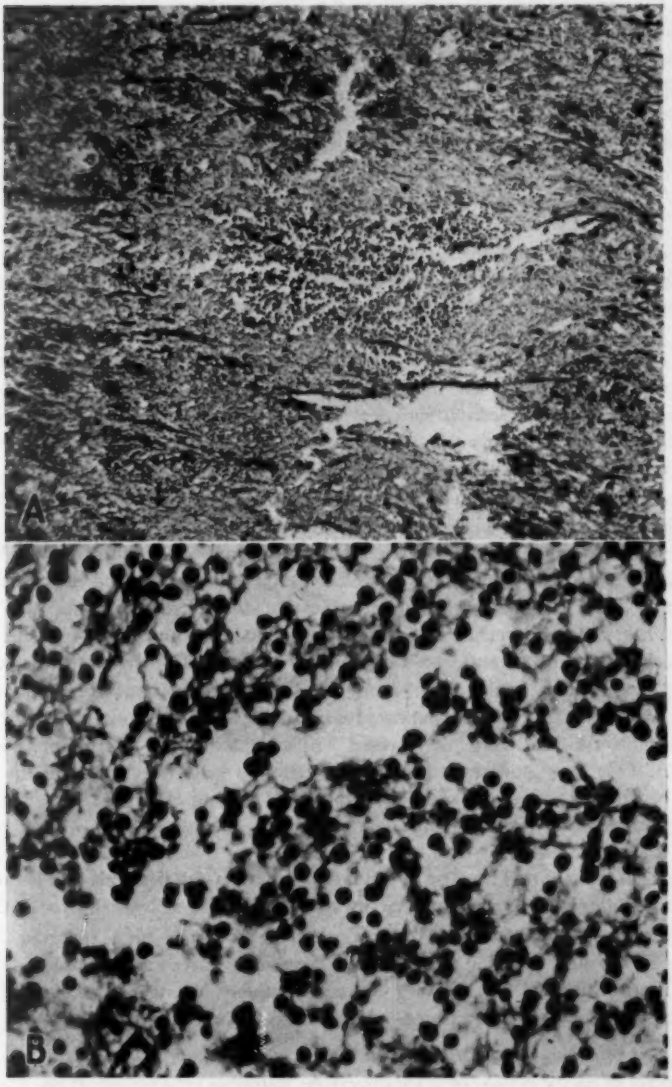


Fig. 2.—Section from pons showing localized infiltration of plasma cells; hematoxylin and eosin; × 70; Armed Forces Institute of Pathology Accession 189106. *B*, higher magnification of same section; × 395.

inferior portion of the left temporal lobe. The lateral ventricles were dilated and filled with blood, and there was dilatation also of the third and fourth ventricles. A small hemorrhagic focus was found in the left branchium pontis. Extensive dissection of the vessels of the middle cerebral artery in the vicinity of the hemorrhage did not yield information as to the exact location of the source of bleeding. No aneurysms were located in this or any of the other arteries of the brain.

The appearance of marrow from ribs, the sternum, and vertebrae was very striking. It was grayish and appeared as if autolysis were taking place. The marrow was diffuse and homogeneous in all bones examined. There was no evidence of bone erosion.

MICROSCOPIC EXAMINATION

The portal spaces of the liver and the central vein areas contained a few more plasma cells than are normally present. The heart had some hyaline, fatty, and calcific changes in the coronary arteries. Some areas showed extravasation of red blood cells within and between the hyalinized intima and the media. In the lungs there was much edema, the alveoli, in places, being widely dilated. The pleura was thickened and fibrotic. The trachea was edematous, and one section showed a chronic inflammatory reaction with loss of mucosal epithelium, the infiltrative cells including numerous plasma cells. Fibrocaseous areas were present in some sections with black-pigment deposits and cholesterol clefts.

Sections of the cerebrum showed large and small parenchymal and small perivascular hemorrhages. The general structure of the spleen was normal, but some of the sinusoids contained numerous plasma cells. One section of the kidney showed a small adenoma, and some of the convoluted tubules had developed early nephrotic changes. Many sinuses in the lymph nodes contained excess plasma cells. One located near a large bronchus had a fibrocaseous nodule with calcific deposits. The stroma of the stomach contained a great many plasma cells. In places the mucosa showed a glandular hyperplasia forming sessile polyp-like structures. There was plasma-cell infiltration in the lamina propria of the large intestine. Marrow from the sternum, ribs, and vertebrae showed a diffuse plasma-cell infiltration (Fig. 1*A* and *B*). One large and several small collections of cells were noted in the pons, many of which were of the plasma-cell type (Fig. 2*A* and *B*).

COMMENT

This case is reported because of the rarity of plasma-cell leukemia and because of the active infiltration of the bone marrow and the absence of bone destruction.

Approximately 36 cases of plasma-cell leukemia have been reported in the literature, the first by Foà [1] in 1902. Of these, only three cases, those of Osgood and Hunter,[2] Piney,[3] and Meyer,[4] showed complete absence of erosion of bone cortex. It is felt that this case adds a fourth to the series.

1. Foà, P.: Sulla produzione cellurale nell' infiammazione ed in altri processi analoghi specialmente in cio che si riferisce alle plasmacellule, abstracted Folia haemat. **1**:166, 1904.

2. Osgood, E. E., and Hunter, W. C.: Plasma Cell Leukemia, Folia haemat. **52**:369, 1934.

3. Piney, A.: Plasma Cell Leukemia, Folia Haemat. **30**:173, 1924.

4. Meyer, L. M.; Halpern, J., and Ogden, F.: Acute Plasma Cell Leukemia, Ann. Int. Med. **22**:585, 1945.

In many ways this case is similar to that recently reported by Staffurth and Spencer,[5] which showed a lymphocytic hyperplasia of the bone marrow of a patient with miliary tuberculosis. This was histologically indistinguishable from lymphatic leukemia. Their patient, however, had active, miliary tuberculosis, and the marrow cells were of the lymphocytic series.

Finally, attention should be called to the fact that hemorrhage presented a prominent aspect in the patient's clinical history and was ultimately the complaint which brought him to the hospital. In addition to hemorrhage involving the central nervous system, which caused his death, the patient had epistaxes, as well as petechiae of the skin and the mucous membranes.

SUMMARY

A case of plasma-cell leukemia in which there were no localized destructive lesions of the bones and in which hemorrhage played an important part is reported.

The Armed Forces Institute of Pathology made the photomicrographs in this case, and Mr. George C. Kidd gave technical assistance.

5. Staffurth, J. S., and Spencer, H.: Aleukemic Leukemoid Response in Miliary Tuberculosis, Blood **5**:1165, 1950.

Special Article

LUDVIG HEKTOEN, PATHOLOGIST
1863-1951

PAUL R. CANNON, M.D.
CHICAGO

AN EVALUATION of the career of Ludvig Hektoen over its more than 60 years of significant contributions to medical science requires an appraisal of its influence both on the development of medical knowledge and on the promotion of human welfare. Fortunately, the task is made less difficult by the fact that his career is represented by a record of sustained achievement seldom equaled. Throughout, it is characterized by a great diversity of interests and a wide range of contributions to medical science and practice. In the record of more than 300 publications one can discern the methods and intellectual traits of an unusual man, many of whose investigations now stand out as landmarks in the history of medicine.

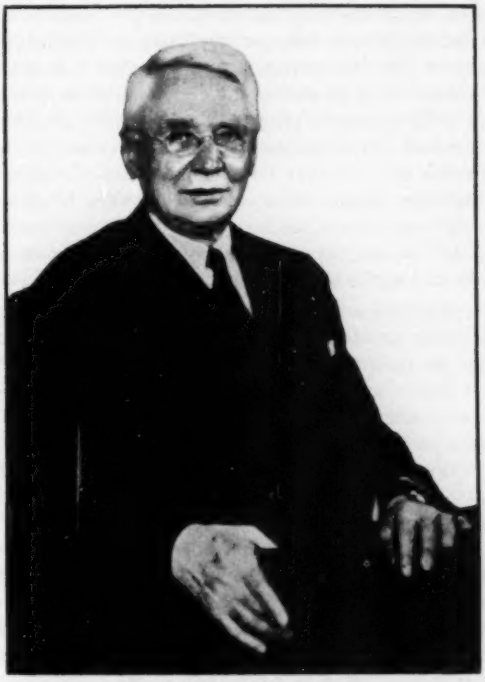
In attempting to evaluate these publications as a whole it is noteworthy that of the first hundred, ending in 1903 with "An Anatomical Study of a Short-Limbed Dwarf," almost all were brief case studies of a type such as any young pathologist might be expected to engage in during his earlier professional years. At this time Hektoen was 40 years of age. One exception is a paper, "The Vascular Changes of Tuberculous Meningitis, Especially the Tuberculous Endarteritis," embodying material collected during time spent in the laboratory of Chiari. This study was the result of an effort to elucidate the pathogenesis of tuberculous meningitis, and the endarterial changes were interpreted as pointing to the hematogenous origin of the meningitis. Another exception to be noted pertains to his studies of some of the fungal infections, particularly actinomycosis, blastomycosis, and sporotrichosis. Nevertheless, inspection of these first hundred publications affords little evidence of the direction of the path which Hektoen was to tread during the next five decades.

In 1901 he became professor and head of the department of pathology and bacteriology at the University of Chicago, and in the following year, director of the newly formed John McCormick Institute of Infectious Diseases. Soon thereafter his scientific communications began to indicate more clearly his interest in a variety of problems related to the subjects of infection and immunity.

In this review an attempt will be made to summarize only his more important contributions in the latter field. In doing so, no attempt will be made to enter into a discussion of priorities with respect to any particular subject; rather emphasis will be placed mainly on the significance of these researches in their relationship to the enlarging knowledge of immunology and infectious disease.

It was between the years 1903 and 1937, in particular, that his most important investigations were carried on. Prominent in all of them, in one way or another, is the problem of antibodies. During this period his active mind led him into many aspects of immune-body reactions, including their nature, sites of formation, modes of action, especially in the animal body, and their usefulness as tools for the

elucidation of many perplexing problems of biology and medicine. As early as 1907, for example, we find him concerned with the phenomena of isoagglutination and blood-group reactions. At that time he pointed out that "the common occurrence of isoagglutinins in human serum suggests that under certain special conditions homologous transfusions might prove dangerous by leading to erythrocytic agglutination within the vessels of the subject transfused." In the present age of blood banks and blood-typing routines it is interesting to note his emphasis on the need for proper blood typing and his cautious acceptance of the seemingly practical



LUDVIG HEKTOEN, M.D.
1863-1951

possibilities of the new procedure of blood transfusions! Soon his name became more and more associated with the subject of antibody mechanisms, and by 1910 he was asked to give a Harvey Lecture. This he did, discussing the question of "The Formation and Fate of Antibodies."

In those early years, just as now, there was much confusion concerning the nature of antibody action. However, there can be no doubt that some of this confusion was lessened materially by Hektoen and his associates. For example, by 1910 he had pointed out the necessity of considering the "antibody curve" as a whole in any study of antibody action, and he had warned against the fallacy of drawing conclusions from a single point on this curve. Even today failure to follow this

basic principle continues to add confusion to the immunological literature. It is noteworthy, also, that Hektoen regarded the antibody curve as an indication of "the balance between production and loss of antibody," thus evidencing his appreciation of the dynamics of antibody action. By this time he had also become interested in the fact that "in animals previously subjected to the action of a certain antigen the mechanism of antibody production may be especially sensitive to that antigen and respond to proper doses more promptly and freely than is the case in fresh animals." This will be alluded to in more detail later. His work with Carlson demonstrated that antigen injected intravenously into dogs is removed from the blood within from 3 to 48 hours, thus indicating that antibodies are produced outside the blood circulation, and that blood itself does not "fix" antigen. He also found that splenectomy just before or just after injection of antigen led to a lower but otherwise typical antibody curve. Later he studied the question of sites of antibody formation by the ablation of different organs prior to or coincident with antigenic stimulation and found that removal of the stomach, the small intestine, or the thyroid gland caused no interference with antibody formation, nor did adrenalectomy or removal of one-half of the liver. From these and other studies he concluded that "the mechanisms for the fabrication of antibodies are quite secure from certain disturbances, and they are in no way contradictory of the current view that these mechanisms are located in the blood-forming organs."

Between the years 1915 and 1922 appeared his important studies dealing with the effects of leucotoxic agents on antibody formation, including x-rays, thorium x, radium emanation, benzene, toluene, and mustard gas. These studies later proved to be especially valuable following the development of the atomic bomb, and in relation to current investigations of radiation injury. In them he observed a marked reduction in output of antibodies after x-radiation, provided that the raying was done several days before the injection of antigen and that it was continued during the period of antibody production. He correlated these findings with the observed facts of a greatly reduced volume of splenic, lymphatic, and thymic tissue, together with changes in the bone marrow, and concluded that his observations harmonized with the view that antibodies are produced in the spleen, the lymphatic tissues, and the bone marrow. He remarked that "the results indicate also that one reason why the lymphocyte appears to be an important agent of defense in tuberculosis and other conditions may be its power to form antibodies." Such chemicals as benzene, toluene, and mustard gas also gave a reduction in the production of certain antibodies, associated with grave lesions in the bone marrow and with leucopenia.

In 1917 he recorded a puzzling observation under the title "Precipitin-Production in Allergic Rabbits." While attempting to produce precipitins to horse protein in rabbits he observed that "the introduction of horse blood or serum in rabbits treated a considerable time previously with sheep blood resulted in the production not only of precipitin for horse protein, but also for sheep protein, as well as for proteins of several other species." As he emphasized, "the particular point on which special stress is placed now is the capacity of the rabbit under suitable conditions . . . to elaborate different precipitins at the same time. It appears that the precipitin-production induced in the usual way leaves behind it an increased power of further production so that large amounts of major as well as group and minor precipitins are elaborated on the injection of a new serum or blood. Manifestly the

phenomenon is an expression of an increased reactivity and may be classed with other manifestations of allergy." This independent observation of what is now known as the anamnestic reaction, aside from its theoretical interest, was seen, also, in its practical implications when he suggested that the reappearance of typhoid agglutinins under the stimulation of other infections could be expected to nullify the significance of the Widal test as a method for the diagnosis of typhoid. He thought of this phenomenon as a "reawakening" of a latent capacity to elaborate specific antibody and pointed to the hazards of such an effect in medicolegal work unless fresh rabbits were used in each instance. Later, with Boor and others, he demonstrated that rabbits receiving simultaneous injections of 35 purified proteins fabricated specific antibodies simultaneously for at least 34 of the 35 antigens injected. Furthermore, when rabbits were immunized against many antigens, the injection later of one of the antigens usually caused the precipitin for that antigen alone to disappear from the blood, indicating that in the rabbit "different precipitins exist as separate entities." He also showed in rabbits in which multiple precipitins had been formed after multiple injections of antigen that at a time when they were no longer demonstrable in the blood many might reappear on the injection of only one of the antigens previously injected.

Hektoen does not seem to have been especially interested in methods, and in much of his work he utilized only the conventional antigen-dilution variety of precipitin test. Today this method is looked on as essentially semiquantitative; indeed, by some, it is regarded as an outmoded serological procedure. Nevertheless, it is remarkable how many complex aspects of immunology he was able to clarify by intelligently applying this comparatively simple method to the solution of specific problems. With it, for example, Hektoen studied such problems as the antigenic components of hemoglobin, of lens protein, of semen, Bence Jones protein, serum proteins, extracts of various animal parasites, thyroglobulin, fibrinogen, egg white, muscle hemoglobin, etc. He was a firm believer in the view advanced by Osborne and Wells of the importance in immunological research of employing purified proteins whenever possible. Associated with him in these studies should be mentioned in particular Welker, Cole, Schulhoff, Whipple, and Robscheit-Robbins. In the studies of lens protein he found a consistent organ-specificness for different species, as he found also in his studies of mammalian thyroglobulins, fibrinogen, and casein. In contrast he observed that solutions of skeletal muscle from the dog were precipitinogenically distinct from those of hemoglobin from dog blood. Of medicolegal significance was his finding that precipitins to human seminal proteins were specific and that this method, therefore, was valuable for the detection of suspected seminal spots and stains, as was the precipitin test for hemoglobin a specific test for blood only. With Welker he concluded that precipitins for several of the blood proteins were individually as well as species distinct, that each protein exists as an independent antigenic unit in the blood or serum and that its antigenic individuality is not the artificial product of the process of separation. With Cole he found that egg white contains five distinct antigens, one of which, conalbumin, was immunologically identical with blood albumin.

Despite his lack of concern for methodology as such, it is important to note that the method developed by Hektoen and Welker for the sustained production of antibodies has been widely used since it was published in 1933. At that time Hektoen

was 70 years of age. This method consisted in the antigen's being adsorbed to aluminum hydroxide prior to its intramuscular injection. They reported that "in rabbits a single intramuscular injection of aluminum hydroxide carrying a measured quantity of antigen may induce continuous formation of precipitin for many months" and that "antigens adsorbed on aluminum hydroxide do not separate on standing and retain their precipitinogenic properties for at least 12 months and probably much longer." By this method it was shown that at least 10 distinct specific antigens may be adsorbed at the same time and such an injected gel will engender the production of 10 specific antibodies.

Besides his continuing interest in basic problems of immunology, Hektoen was also interested in the pathogenesis of measles, and as early as 1905 he had produced the disease experimentally in human subjects by the subcutaneous injection of blood from a measles patient, thus demonstrating that the virus may be in the blood at least during the first 30 hours of the rash. In 1911, with Eggers, he also reported the production of measles in rhesus monkeys by injection of citrated measles blood. He maintained a keen interest, also, in the problem of scarlet fever, and contributed to the studies of Tunnicliff, and of George F. and Gladys Henry Dick, in their significant contributions to the better understanding, control, and treatment of this disease. He was also a pioneer contributor to our knowledge of coronary thrombosis and performed the necropsy on a patient reported on by Herrick in 1912. In this patient Hektoen described the presence of a red thrombus which had completely occluded the sclerotic left coronary artery, accompanied by severe myocardial infarction and a nonbacterial fibrinous pericarditis.

When one takes into account the fact that these investigations were made over a great span of years by a man busily engaged in an extraordinary variety of practical enterprises, including the editing of the *Journal of Infectious Diseases* and the ARCHIVES OF PATHOLOGY, as well as functioning as a leader in national medical affairs, it is evident that his ability to organize his daily work must have been truly remarkable. He received many honors at home and abroad, was twice Chairman of the Medical Division of the National Research Council, was active in the National Academy of Sciences, in medicolegal affairs, and in various phases of the study of neoplastic diseases. Through it all he remained a kindly and patient man, modest and beloved. As Fishbein said of him, he was "the possessor of a calm gentility." He inspired many associates in many ways, but his own record, now "fixed in print" is objective proof of his own scientific bequest to medical science and human betterment.

Hektoen always had a great respect for history and tradition. Many of his own contributions are now themselves a part of the history of medicine. Moreover, by precept and example he helped to establish traditions in pathology which will serve as guides to other pathologists today and in the years to come. Among these should be noted in particular his insistence on a clear and direct style of medical writing. In addition he demonstrated the cumulative value of such attributes as persistence, sustained industry, the intelligent application of simple methods to specific problems, a meticulous devotion to duty, and a life-long enthusiasm for his chosen fields of interest. Because of these and other qualities his contributions to the history and traditions of pathology and medicine will long command the emulation and respect which they so well deserve.

Notes and News

Catalog of Medical Motion Pictures.—A revised catalog of medical motion pictures is now available through the Committee on Medical Motion Pictures of the American Medical Association. Copies will be sent to the secretary of each county and state medical society. This catalog lists sixty-two 16-mm. films, most of which are at the professional level. Fourteen of these films are suitable for showing to lay groups. Eight new films have been added. Copies are available on request from: Committee on Medical Motion Pictures, American Medical Association, 535 N. Dearborn St., Chicago 10.

Deaths.—Dr. Malcolm H. Soule, professor of bacteriology and head of the department at the University of Michigan since 1935, died Aug. 3, 1951. He attained wide recognition for his contributions in the field of bacteriology and was a member of the leading societies in this specialty. Also, he was a member and former president (1947) of the American Association of Pathologists and Bacteriologists. In recent years he served as assistant editor of the *American Journal of Pathology.*

Books Received

THE MICROKARYOCYTES: THE FOURTH CORPUSCLES AND THEIR FUNCTIONS. By K. G. Khorozian, M.S., M.D., Pineville, W. Va. Cloth. Pp. 969, with 480 illustrations. Price $12. Meador Publishing Company, 324 Newbury St., Boston 15, 1951.

This book attempts to revolutionize the entire present concept of normal and morbid cellular morphology and physiology by creating a newer concept based on the microkaryocytes, the cells with the smallest nuclei so far known to exist. The author claims that the physiological properties, physical characters, composition of the blood, tissue cells, and the entire structural make-up of all living organisms, both animal and plant, are dependent on, and cytologically composed of, microkaryocytes. In size they are equal to the granules seen in leucocytes, although these granules are supposedly the microkaryocytes themselves. They measure from 0.15 to 0.2 micron in diameter, and from 35 to 60 of these cells can be demonstrated in a single red blood cell. By a crude technique of computation, the author comes up with 8,960,000,000 microkaryocytes per cubic millimeter of plasma. The original source of these hitherto unknown corpuscular elements is the yellow bone marrow, probably the endothelial lining of the capillaries of the marrow. The entire premise of this work and the demonstration of these cells are based on what the author calls the microkaryocytic techniques, which utilize ordinary and special staining techniques, photomicrography, and high photographic enlargements, as much as 25,000 times. With these methods "dot-like looking bodies, with dark centers and refractile surroundings as a halo" were observed by Dr. Khorozian. He excludes the notion that these tiny bodies may represent some outside contamination, or some artefact, or that they may be blood platelets. All the physiological processes in the body, such as carbohydrate, protein, and fat metabolism, the mechanism of iron metabolism, the products of both the glands of external and the glands of internal secretion, the hepatic and pancreatic functions, the defense mechanisms of the body, including antibodies, the human testicular and ovarian structures, as well as the endometrium in its nongravid and gravid cycles, and the nourishment of the embryo and of the fetus, are structural and functional units of the microkaryocytic mechanism. The dissemination and therapeutic utilization of all therapeutic agents are accomplished by these cells. The cancer cell is supposedly composed of microkaryocytic cells in which can be demonstrated the existence of certain bodies which the author believes are the causative agent of cancer or the virus of cancer.

Many of the most valuable contributions made to medical science in the field of biochemistry, nutrition, and colloidal and physical chemistry, which are based largely on observations of a highly specialized and technical nature, are either pooh-poohed and cynically passed over as not being based on demonstrable observations and factors or are labeled misconceptions and misinterpretations. Scientific investigators and editors are taken to task for not having accepted his discovery as an "epoch of epochal dates in medicinal and biological history." This book is filled with theoretical speculations and hypotheses. The superman attitude of the author in addition to his verbose enthusiasm and repetitious, dogmatic statements, which lack any semblance of judicious control, renders this book worthless as a scientific piece of work. The extent to which hypertrophy of imagination can be taxed in order to make theoretical speculation appear factual is exemplified in this book. The illustrations, owing to their high photographic enlargements, not only are poor but lack definition and have a cloudy appearance. The money that was expended in the publication of this book could have been used for publishing a really worthy scientific work.

TUMORS OF THE ADRENAL. By Howard T. Karsner, M.D., medical research adviser, United States Navy, Bureau of Medicine and Surgery; formerly professor of pathology, Western Reserve University. Atlas of Tumor Pathology, Section VIII, Fascicle 29, Pp. 59, with 47 illustrations and 5 colored plates. Price $1. Armed Forces Institute of Pathology, Washington, D. C., 1950.

This is the second chapter of the projected atlas to be released and here reviewed. The tumors of the adrenal cortex and medulla are presented by many illustrations, brief descriptions, and a few references. Any atlas sponsored by such an impressive list of organizations as stands behind this, that is, so apparently well financed, and which is published for the purpose of unifying nomenclature ought to be nearly perfect. This chapter is not. Although it appears good in some respects, it shows deficiences in illustration, layout, and editing, and it contains a few errors in statement of fact.

A number of the illustrations show artefacts and some show the effects of failure of proper fixation. Figures 5 and 37 are of poor technical quality, being out of focus. Figure 5 is upside down, according to the legend. Figures 16, 17, 18, 19, 20, 31, 32, 38, 39, and 41 are wasteful; each occupies half a page when one-quarter page or less would have done just as well because they are high-power photomicrographs of fairly uniform tissue in which so much duplication of cells is not necessary. The interpretation given for Figure 16 is challenged; serial sections might well show that this is coiled fasciculata and not glomerulosa as stated. Many figures are essentially duplicates and add little or nothing new; examples of duplication are Figures 7, 8, 10, 11, 12, and 13, Figure 9 and Plate 1A, and Figure 36 and Plate IVB. Poor teaching psychology is used in showing tumor metastases before the tumor as in Figures 23, 24, and 25. Plate IV has striking colors but little scientific information. Cell invasion of the capsule is frequently used as a point of distinction between cortical carcinoma and adenoma; yet Figure 21, illustrating the former, shows less capsular invasion than does Figure 3, that of a "cortical nodule." In this connection, on Page 7, it is erroneously stated that when lipids are reduced in amount in the cortex they usually remain in the nodules. The "cortical nodule" is not clearly designated for what it is, namely, a part of nodular hyperplasia. When nodules are common, they are less common than the less perfectly formed nodular areas, and all intermediate stages are frequent.

This fascicle is recommended with more reservations than the first. Subsequent chapters ought to be greatly improved if the atlas is to accomplish its aims and be a credit to its sponsors.

LA TALASSEMIA. By Drs. G. Astaldi, P. Tolentino, and C. Sacchetti, of the Institute of Clinical Medicine and Clinical Therapy, of the University of Pavia, and of the Institute of Clinical Pediatrics, of the University of Genoa. Biblioteca "Haematologica" XII. Paper. Pp. 229, with 69 figures and 17 tables. Price 2500 lira ($4). Tipografia del Libra, Pavia, Italy, 1951.

This monograph is an important contribution to the subject of thalassemia. The first seven chapters of the first part of the book are concerned with the history and genetics of the disorder, followed by the etiology and the symptomatology, including clinical and radiologic aspects of the disease. The hematologic signs, the bone marrow picture, and the pathologic findings are covered in detail. The authors correlate three forms of thalassemia, each distinguished from the other by clinical, roentgenologic, hematologic, and other laboratory tests:

1. Thalassemia major, or Cooley's disease, is always of homozygotic origin. The more serious form begins at a very early age (in 2- to 3-month-old infants). The blood findings are easily confused with erythremic or erythroleukemic myelosis when the background and the case history of the parents are not known. The second type of the major form, which generally begins in the second half of the first year, has a longer duration than the first, and its clinical picture is the best known. The third type of the major form occurs in adults. It is less severe than the other two and begins after the second year. Its evolution is longer, because it can last up to adulthood. The hematologic alterations, however, can be intense even in this form. The finding of fetal hemoglobin is considered of diagnostic importance in doubtful cases.

2. Thalassemia minor is a heterozygotic condition which is compatible with continuance of life.

3. Thalassemia minima or microcytemia is also a heterozygotic condition. The condition, although not serious for the patients, is definitely risky for their children, who may become afflicted with the minima, the minor or the major form of the disease, depending on whether one of the parents is a healthy individual or both have the disease.

The second part of the monograph, consisting of six chapters, is devoted to a comprehensive study of the pathologic physiology and the biologic properties of the erythropoietic tissue of the patient with thalassemia, based on the technique of marrow culture in vitro. Each of these chapters is so replete with significant observations and deductions that it is difficult to single out subjects for review. Cellular differentiation was studied by keeping the surviving marrow cells in a Carrel flask containing a liquid medium consisting of plasma and Tyrode solution. Studies on the proliferative ability of the erythroblasts were carried out by means of fragments of marrow explanted into a solid medium containing colchicine in the optimum concentration. By these methods the activity of the thalassemic erythroblasts was compared with normal standards. By means of experiments carried out with "crossed" plasmas (thalassemic erythroblasts in normal plasma; normal erythroblasts in thalassemic plasma) it was inferred that the erythropoietic defect and shorter life span of the thalassemic erythrocyte are closely linked to its intimate biologic nature and do not depend on the plasmatic circumambient in which the cell is living. These investigators conclude from their clinical and experimental research that the morphologic and biologic anomalies of the erythrocyte in thalassemia are primarily constitutional medullary defects, the first one being due to a gradual reduction of erythroblastic cytoplasm during the maturation of hemoglobin and the second one to an erythroblastic flaw that entails the formation of labile erythrocytes; the end result is erythrocytes that are born already old.

Paper, printing, illustrations, and photomicrographs are good. There is a clear, concise 12-page summary in English. The bibliography contains over 400 references. This excellent monograph should be in all medical libraries and in the collection of all specialists on blood.

SURGICAL FORUM : PROCEEDINGS OF THE FORUM SESSIONS, THIRTY-SIXTH CLINICAL CONGRESS OF THE AMERICAN COLLEGE OF SURGEONS, BOSTON, OCTOBER, 1950. Pp. 665, with 260 illustrations and 6 tables. Price $10. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London W.C. 2, 1951.

The purpose of this book is to present in one volume the papers read at the Forum on Fundamental Surgical Problems at the meeting of the American College of Surgeons in October, 1950. Since their inception in 1941 these sessions during which reports of research in the various fields of surgery are presented have been among the most stimulating and thought-provoking meetings of the annual clinical congresses of the American College of Surgeons.

The volume presents papers covering research in all fields of general surgery and also neurosurgery and anesthesia. The articles, in general, are well written and adequately illustrated, and they present the material succinctly. Unfortunately, 58 of the 164 papers are represented only by short abstracts, and the book thereby loses some of its value.

This collection of papers should be read not only by all interested in experimental surgery and physiology but by all practitioners who have an interest in the scientific progress of surgery. Its appearance only a few months after the presentation of the papers is a major accomplishment on the part of the publishers. One hopes that the response to this volume will be so favorable that it will be possible in future years to present all papers in full.

THE KIDNEY-MEDICAL AND SURGICAL DISEASES. By Arthur C. Allen, M.D., pathologist, The James Ewing Hospital; assistant attending pathologist, Memorial Cancer Center, New York; attending consultant in pathology, Veterans Administration Hospital, Bronx, N. Y. Pp. 583, with 1,115 illustrations. Price $15. Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1951.

This author has presented the subject of the diseases of the kidney in a very attractive way. One principal consideration is a correlation of disturbances in function and alterations in structure. The structural changes encountered in disease are thoroughly described in the text and superbly illustrated. The relationship borne by these changes to functional derangements is evaluated with caution and understanding. The author has carefully expressed his opinions throughout the text, but an adequate bibliography representing opinions of other authors concludes each chapter. For the past several years our attention has ben drawn to so much chemistry and physiology of abnormal renal function that many of us have almost forgotten that the functional units of the kidney are ever structurally impaired. This book, written by a pathologist, serves to remind us that function and structure are almost inseparable. An understanding of the one requires a thorough understanding of the other. Where discrepancies exist, and there are many, further study should resolve them. There is no better monograph on diseases of the kidney than this one.

KLINISCHE PHYSIOLOGIE UND PATHOLOGIE. By Prof. Dr. Ferdinand Hoff, Chief of the Medical Clinic of the City Hospitals in Aachen. Linen. Price, 39.00 german marks. Pp. 782, with 124 illustrations. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart-O, 1950. Agents for U. S. A., Grune & Stratton, Inc., 381 4th Ave., New York.

This book represents an attempt to unify physiology, clinic and pathology and to emphasize the fundamental oneness of physical and psychical phenomena. Extreme specialization in medicine has been responsible for enormous accumulation of isolated observations, many of which remained detached, neither correlated with one another nor with the body as a whole. Thus one has come to think of the liver, the heart, the kidney, etc., and of their disorders as separate and distinct entities, whereas actually they do not exist as such, but are always dependent, more or less, on the other organs. Hoff tries to counteract this tendency, and he is doing it quite successfully. Especially impressive is the emphasis on the influence of the central nervous system on all the functions of the body, normal and abnormal. The introductory and closing chapters contain an interesting and stimulating philosophic discussion on the nature of disease. Somewhat similar is chapter 11 which deals with regulatory mechanisms, especially the role of the nervous system. The other 11 chapters cover the heart and vascular system, the respiratory organs, the blood, the liver and biliary tract, the pancreas, the gastrointestinal tract, the kidneys, organs of locomotion, the endocrine glands, and metabolism.

This is a unique book, rich in information, original in point of view and presentation, well written, and most stimulating. The illustrations are well chosen and executed; the binding, paper, and print are excellent.

METABOLISME DU FER: PHYSIOLOGIE, PATHOLOGIE, TRAITEMENT. By G. Hemmeler. Pp. 258, with 47 illustrations. Price 1,500 francs. Masson & Cie, 120 Boulevard Saint-Germain, Paris, 6e, 1951.

PRINCIPLES AND PRACTICE OF OBSTETRICS (Originally by Joseph B. DeLee, M.D.). By J. P. Greenhill, M.D., attending obstetrician and gynecologist, Michael Reese Hospital; obstetrician and gynecologist, Associate Staff, Chicago Lying-In Hospital; attending gynecologist, Cook County Hospital; professor of gynecology, Cook County Graduate School of Medicine. Tenth edition. Pp. 1020, with 1,140 illustrations on 864 figures, 194 in color. Price $12. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London W.C. 2, 1951.

TREATMENT OF THE NEPHROTIC SYNDROME. By Lee E. Farr, M.D., chairman, Medical Department, Brookhaven National Laboratory; physician-in-chief, Brookhaven National Laboratory Hospital, Upton, Long Island, N. Y. Pp. 61. Price $1.75. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1951.

STUDIES IN PATHOLOGY. Presented to Peter MacCallum. Edited by E. S. J. King, T. E. Lowe, and L. B. Cox. Pp. 350, with 48 illustrations. Price $7. Cambridge University Press, Bentley House, 200 Euston Rd., London, N.W. 1; American Branch, 51 Madison Ave., New York 10, 1950.

This volume is dedicated to Prof. Peter MacCallum, an eminent Australian pathologist. There are 23 contributors, representing his former students and associates. Eleven of these are actively engaged in the field of pathology in the Melbourne area. Two are distinguished pathologists in London. One is an American, who was honorary lecturer in pathology at the University of Melbourne during World War II and is now Morrison professor of pathology at Northwestern University in Chicago.

The topics are sufficiently varied so as to constitute almost a cross-sectional study of the field of morbid anatomy. There are studies on the problems of general pathology, such as the contribution of pathology to biology, angiogenesis, metaplasia, infarcts, the location of glycogen in liver cells and lobules, and observations on hemosiderosis. The field of special pathology includes a discussion of normal and pathologic patterns in the liver, myocardial infarction, vascularization and hemorrhage in the arterial wall, the general changes in rheumatoid arthritis, phases of tuberculosis in childhood, necrosis in Hodgkin's disease, the surgical pathology of esophageal cancer and of carcinoma of the lung, and chocolate cysts of the ovary. There are two chapters which deal with the lymphoid apparatus. The embryological relationship of maternal rubella and congenital heart disease is discussed in one chapter. There are also contributions in the field of endocrinology, including intersexuality and diabetes insipidus. Lastly there is one paper of medical-legal interest, viz., the examination of blood and stains in crime detection.

The style, illustrations and format of this volume are a tribute to the man who is honored. Each chapter is interesting reading. No matter how outworn a title may appear, there are new angles and new problems that are brought to bear on the subject, which stimulate and excite the imagination. The editors and the publishers are to be commended for a worth-while contribution to the field of pathology.

THE QUANTITATION OF MIXTURES OF HEMOGLOBIN DERIVATIVES BY PHOTOELECTRIC SPECTROPHOTOMETRY. By Francis T. Hunter, A.M., M.D., associate in medicine, Harvard Medical School; associate physician and clinical pathologist, Massachusetts General Hospital, Boston. Pp. 226, with a number of charts and figures. Price $8.50. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill.

FROM A DOCTOR'S HEART. By Eugene F. Snyder, M.D. Pp. 251, with numerous drawings. Price $3.75. Philosophical Library, Inc., 15 E. 40th St., New York 16, 1951.

REVIEW OF PHYSIOLOGICAL CHEMISTRY. By Harold A. Harper, Ph.D., professor of biology (biochemistry), University of San Francisco; lecturer in surgery, University of California School of Medicine, San Francisco; biochemist consultant to Metabolic Research Facility, United States Naval Hospital, Oakland; director, Biochemistry Laboratory, St. Mary's Hospital, San Francisco. Third edition. Pp. 260, with 13 figures. Price $3.50. University Medical Publishers, Palo Alto, Calif., 1951.

CLINICAL PEDIATRIC UROLOGY. By Meredith Campbell, M.S., M.D., F.A.C.S., professor of urology, New York University Post-Graduate Medical School, and visiting urologist, Bellevue and University Hospitals, New York. Pp. 1113, with 543 illustrations. Price $18. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1951.

This new work of Dr. Campbell is excellent, as was his previous two-volume work. The chapters on examination and diagnosis are very complete. The book covers all phases of urological disease and practice, with the exception of diseases of the adult prostate. Throughout

the book, of course, the treatment of each subject is focused on the conditions in the infant and child. With comparatively few exceptions, however, the basic physiological and pathological principles of both diagnostic procedures and modes of treatment are directly applicable to the same diseases in the adult. The sections on embryology and anomalies are naturally most complete. There is a short but excellent review of urological surgical procedures. The book is a practical one and stresses procedures useful in ordinary practice. The author's immense experience with pediatric urological diseases gives his opinion a great deal of weight. That urological disease in children is now being recognized much more frequently and corrected much earlier in life is due to a great extent to Dr. Campbell's efforts.

Every practicing urologist and pediatrician should have this work. The format of the book is good, and the illustrations are numerous and well reproduced. There is a good index. These features have undoubtedly contributed to the rather formidable price ($18). The book is too long (1,100 pages) for a textbook, and the emphasis it lays on pediatrics also makes it unsuitable for this purpose, but in its own field it has no rivals.

PAPAIN. By M. L. Tainter and 12 other authors. Pp. 153, with numerous tables and figures. Price $3. Annals of the New York Academy of Sciences, Vol. 54, Art. 2, pp. 143-296. Published by the Academy of Sciences, New York, May 16, 1951.

IMMUNOLOGY. By Noble Pierce Sherwood, Ph.D., M.D., professor of bacteriology, University of Kansas, and pathologist to the Lawrence Memorial Hospital, Lawrence, Kan. Third edition. Pp. 731, with numerous illustrations. Price $8. C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, 1951.

In reading this book, one cannot avoid noticing the omission of many of the most recent advances in immunology. The complete neglect of the quantitative studies on precipitation, agglutination and complement-fixation reactions is a serious shortcoming of the book. The emphasis which the author places on the older literature and the historical facts on the subjects treated is striking. This, of course, can be considered as one of the strong points of the book.

The style in which this book is written seems a bit cumbersome. To a large extent this is due to the division of each chapter into innumerable subheadings which in many instances appear unnecessary. The organization of the subjects discussed in some chapters leaves much to be desired.

There are various chapters which are well treated. Of these the one on modified and conjugated antigens and those dealing with hypersensitivity are recommended.

It is felt that the main value of this book is its well documented presentation of the older literature of most of the subjects treated.

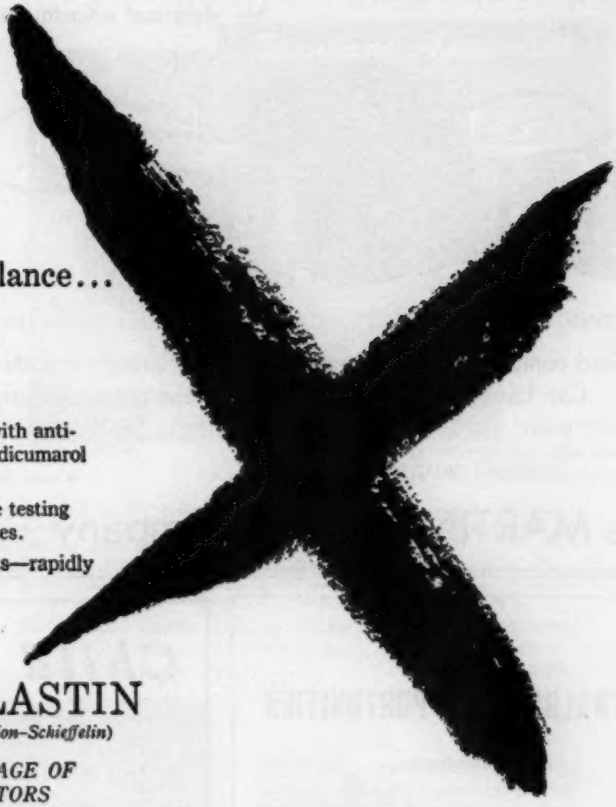
PRIMARY CARCINOMA OF THE LIVER: A STUDY IN INCIDENCE, CLINICAL MANIFESTATIONS, PATHOLOGY AND AETIOLOGY. By Charles Berman, M.D., B. Ch. Rand, senior medical officer, Consolidated Main Reef Mines and Estate, Ltd., Maraisburg, Transvaal, South Africa. With a Foreword by Sir Ernest Kennaway, M.D. Oxon., D.Sc. Lond., late director of Chester Beatty Research Institute, Royal Cancer Hospital, London; professor emeritus of experimental pathology at the University of London. And an Introduction by Henry Gluckman, M.R.C.S. Eng., L.R.C.P. Lond., former Minister of Health, Union of South Africa. Pp. 164, with 83 illustrations. Price 35 shillings net. H. K. Lewis & Co., Ltd., 136 Gower St., London, W.C. 1, 1951.

Primary carcinoma of the liver presents a problem of considerable importance in parts of Africa and Asia. Its prevalence in these areas is related to genetic and environmental factors which appear to play an important role in the pathogenesis of the disease. Dr. Berman's excellent monograph, the result of more than 15 years work in the field, covers quite completely the historical, clinical and pathologic aspects of carcinoma of the liver. Brief chapters deal with the role of cirrhosis and of cirrhosis-producing agents, as well as with the exogenous and endogenous (tissue extracts) carcinogenic agents used in the experimental production of primary

cancer of the liver. Clinically the author recognizes five main patterns of the disease: frank cancer, acute abdominal cancer, febrile cancer, occult cancer and metastatic cancer. Pathologically, hepatocellular and cholangiocellular types of carcinomas are described. This precise nomenclature deserves general acceptance. The book is without doubt the most complete work on the subject. Eighty-three well chosen illustrations and more than 500 references should add to its value.

CLINICAL AND ROENTGENOLOGIC EVALUATION OF THE PELVIS IN OBSTETRICS. By Howard C. Moloy, M.D., M.Sc., assistant clinical professor of obstetrics and gynecology, College of Physicians and Surgeons, Columbia University, and the Sloane Hospital for Women. Pp. 119, with 68 illustrations. Price $2.50. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1951.

SPEZIELLE PATHOLOGIE DER KRANKHEITEN DES ZENTRALEN UND PERIPHEREN NERVENSYSTEMS. By Dr. Gerd Peters, professor of neurology and psychiatry, University of Bonn, Germany. Pp. 437, with 214 illustrations. Price 56 German marks. George Thieme, Diemershaldenstrasse 47, (14a) Stuttgart-O; Agents for U. S. A., Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1951.



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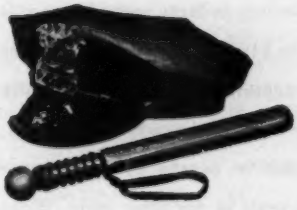

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